Selectivity Comparison of Agilent Poroshell 120 Phases in the Separation of Butter Antioxidants

Application Note

Food Testing & Agriculture

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Abstract

The selectivity of various phases of Agilent Poroshell 120, including EC-C18, SB-C18, SB-Aq, Bonus-RP, and Phenyl-Hexyl, were compared in the separation of nine artificial antioxidants. The method was then optimized on an Agilent Poroshell 120 SB-Aq column for these compounds in butter samples with a water (with acetic acid modifier) and acetonitrile mobile phase. The method separated the antioxidants very well and was suitable for quantitative analysis.

Introduction

Foods, such as oils and fats, containing unsaturated fatty acids can easily cause lipid oxidation leading to rancidity, odor problems, and a decrease of their nutritional value. Synthetic ascorbyl palmitate and phenolic antioxidants are often added to foods to prevent oxidation of these unsaturated fatty acids. Single or combinations of antioxidants are permitted to enhance the antioxidative effect in food, but excessive consumption can cause some health problems in humans. For example, 2,4,5-trihydroxybutyrophenone has mutagenic effects, butylated hydroxyanisole and 2,6-di-tert-butyl-4-methylphenol may be carcinogenic, and propyl gallate can cause kidney damage [1].

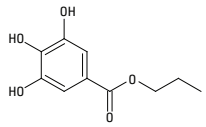
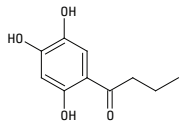
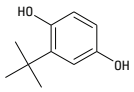
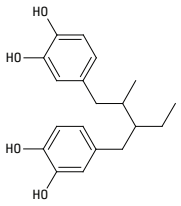
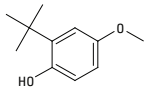
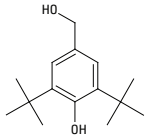
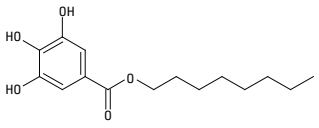
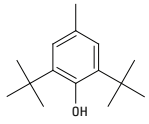
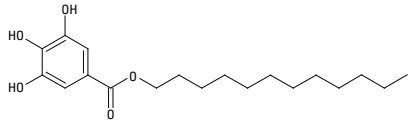
Regulatory agencies from both Europe and the US have imposed maximum levels for some antioxidants while others have been forbidden [2]. Therefore, the determination of antioxidants in foods and food components is important. Table 1 lists nine antioxidants that are most commonly added to foods along with their structures and abbreviations.

Currently, a regulatory HPLC method in China is used for the determination of antioxidants in fats. This method uses columns 15 to 25 cm long with an internal

diameter of 4.6 mm, packed with 5 µm C18 bonded silica particles, and a mobile phase composed of acetic acid (eluent A) and methanol (eluent B). Unfortunately, this method is quite slow with an analysis time over 30 minutes [3]. QC laboratories in fat-processing industries are looking for a way to shorten analysis time, because some of the antioxidants have been found to suffer significant degradation in solvents at room temperature. Thus, a fast, accurate, and rugged method is desirable for economical and practical reasons.

This application note describes the analysis of nine antioxidants in butters using the Agilent 1290 Infinity LC with Poroshell 120 columns. Selectivity of various phases was compared for separation. The Poroshell 120 SB-Aq column was chosen for method development for its better selectivity and retention time versus other phases, and then the method was optimized to fit the analysis of various butter samples.

Table 1. Artificial antioxidants used in this study.

Peak No.	Name	CAS	Structure
1	Propyl gallate (PG)	121-79-9	
2	2,4,5-Trihydroxybutyrophenone (THBP)	1421-63-2	
3	2-Tert-butylhydroquinone (TBHQ)	1948-33-0	
4	Nordihydroguaiaretic acid (NDGA)	500-38-9	
5	Butylated hydroxyanisole (BHA)	25013-16-5	
6	Ionox-100	88-26-6	
7	Octyl gallate (OG)	1034-01-1	
8	2,6-Di-tert-butyl-4-methylphenol (BHT)	128-37-0	
9	Dodecyl gallate (DG)	1166-52-5	

Materials and Methods

HPLC analysis was performed with the Agilent 1290 Infinity LC, including an Agilent 1290 Infinity Binary Pump (G4220A), an Agilent 1290 Infinity Autosampler (G4226A), an Agilent 1290 Infinity Thermostatted Column Compartment (G1316C), and an Agilent 1290 Infinity Diode Array Detector (G4212A).

Columns

Agilent Poroshell 120 EC-C18, 3.0 × 100 mm, 2.7 µm (p/n 695975-302)

Agilent Poroshell 120 SB-C18, 3.0 × 100 mm, 2.7 µm (p/n 685975-302)

Agilent Poroshell 120 SB-Aq, 3.0 × 100 mm, 2.7 µm (p/n 685975-314)

Agilent Poroshell 120 Bonus-RP, 3.0 × 100 mm, 2.7 µm (p/n 695968-301)

Agilent Poroshell 120 Phenyl-Hexyl, 3.0 × 100 mm, 2.7 µm (p/n 695975-312)

The stock solution of standard's mixture was prepared in methanol at 0.1 mg/mL individually. The standard solutions for linearity were diluted from the stock solution in a series of concentrations including 0.2, 0.5, 1, 2, 5, and 10 ppm in acetonitrile:isopropanol (50:50 v:v).

The butters were purchased in the supermarket, and originated from the US, Switzerland, Australia, and New Zealand. Sample solutions were prepared according to the Chinese regulatory method [3]. For the spiked samples, a stock solution of the antioxidants in the solvent was added prior to extraction. The extraction was carried out by weighing 1.0 g of butter and adding 5.0 mL of *n*-hexane saturated with acetonitrile. This mixture was vortexed to dissolve the butter, was added to 10 mL of acetonitrile saturated with *n*-hexane, was vortexed again for 1 minute, and was allowed to stand until a separate layer developed. The acetonitrile layer was collected and extracted once more with acetonitrile saturated with *n*-hexane. The collected acetonitrile portions were mixed together and concentrated with a flow of nitrogen to a volume of 1 mL, and then made up to 2 mL with isopropanol. These solutions were filtered using Agilent 0.2 µm regenerated cellulose membrane filters (p/n 5064-8222) before transfer into autosampler vials for injection.

Results and Discussion

Selectivity comparison

Poroshell 120 columns were packed with superficially porous particles, which provided performance similar to the sub-2 µm particles but with a 40 to 50% lower pressure than columns with sub-2 µm particles. The recent introduction of new stationary phases available on Poroshell 120 columns made them useful for method development by changing selectivity.

Using a variety of bonded phases to try sequentially for method development demonstrated the different selectivity easily gained from the columns. Figure 1 and Figure 2 are overlays of five different reversed phases with acetic acid:methanol and acetic acid:acetonitrile mobile phases. All gave a symmetrical peak shape, with the exception of the Poroshell 120 Bonus-RP with methanol organic phase. This might have been caused by the amide group embedded in the bonded phase of Poroshell Bonus-RP, which has a strong H-bonding attraction with acidic analytes when methanol is present in the mobile phase.

The differences in selectivity between the five columns were due to the differences in bonding chemistry, such as the type of bonding, the end capping, and the amount and type of silanols on the silica surface. Other factors that influence selectivity, including mobile phase composition, temperature, and pH were identical during the investigation. These five bonded phases were all based on 2.7-µm Poroshell 120 superficially porous silica. They included an EC-C18 column, highly end capped that gave the best overall peak shape; SB-C18 and SB-Aq non-end capped columns, which had interaction with silanol groups and provided alternative selectivity to C18 phases; Bonus-RP, a polar embedded amine column that gave unique selectivity; and a Phenyl-Hexyl bonded column, which had improved selectivity for aromatic compounds.

Under a mobile phase of acetonitrile/1.5% acetic acid, the Phenyl-Hexyl and EC-C18 did not resolve peaks 6 and 7 well . SB-C18 and SB-Aq both resolved all nine peaks, but SB-C18 gave longer retention and less resolution between peaks 6 and 7 than SB-Aq. When changing the mobile phase to methanol:1.5% acetic acid, the elution order changed on all columns with poor separation for several peaks, but SB-Aq still separated all nine compounds well. In a careful comparison between the two mobile phases on the SB-Aq column, acetonitrile:1.5% acetic acid gave even better resolution and higher performance for all the compounds. Therefore, the Poroshell 120 SB-Aq was chosen for further method development with the mobile phase of acetonitrile:1.5% acetic acid.

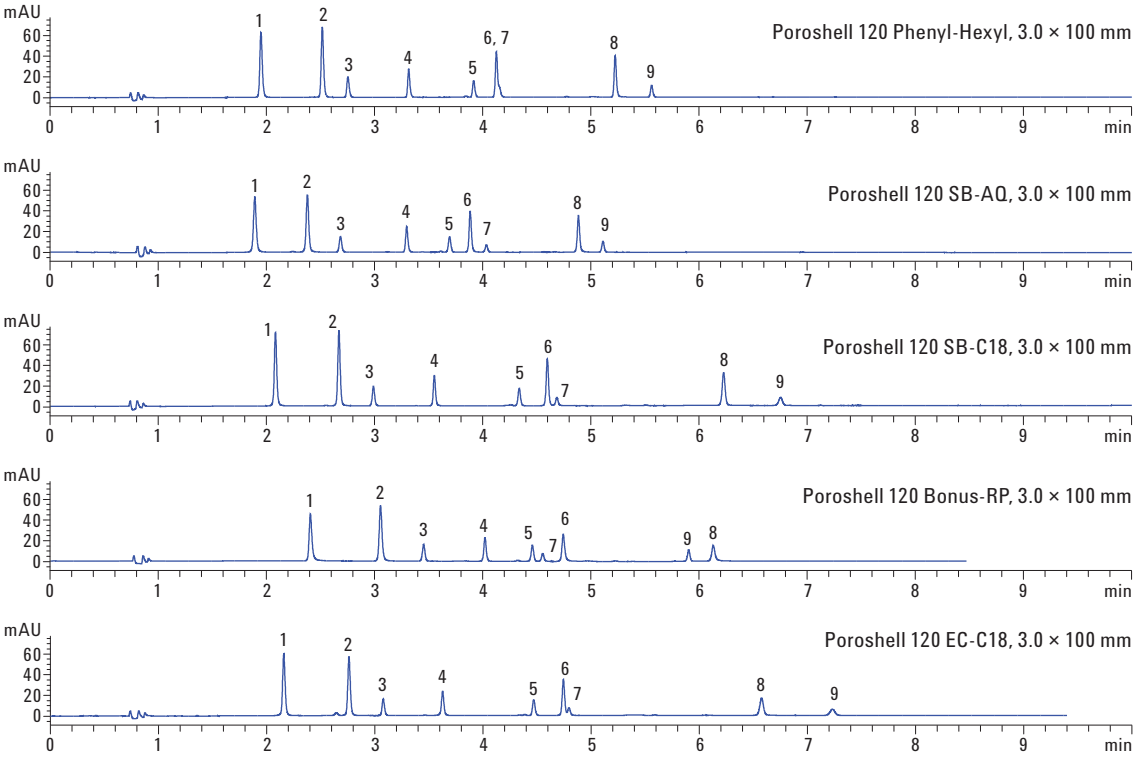


Figure 1. Chromatograms of nine antioxidants in acetonitrile:1.5% acetic acid on various Agilent Poroshell 120 phases showing different selectivity.

Conditions, Figure 1

Eluent:	A 1.5% acetic acid, B ACN	
Injection volume:	2 µL of 10 ppm mixture in 10% methanol	
Flow rate:	0.6 mL/min	
Gradient:	Time (min)	% B
	0	25
	0.5	25
	5	80
	8	80
Temperature:	40 °C	
Detector:	UV, 280 nm	

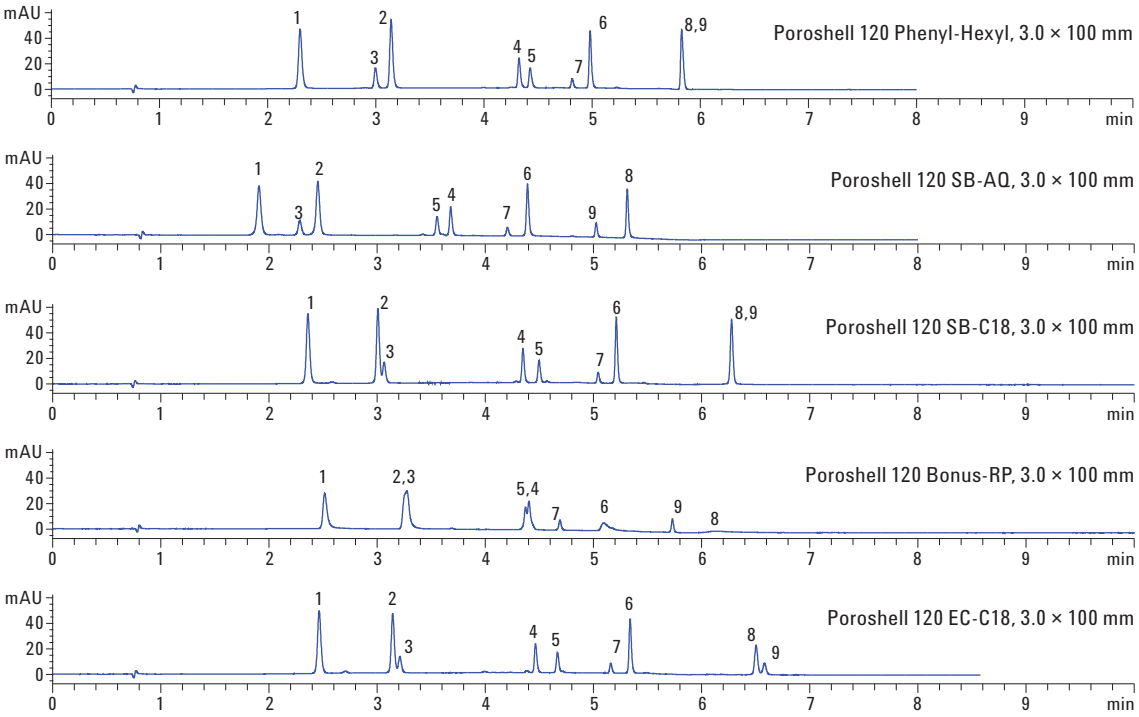


Figure 2. Chromatograms of nine antioxidants in methanol:1.5% acetic acid on various Agilent Poroshell 120 phases showing different selectivity.

Conditions, Figure 2

Eluent:	A 1.5% acetic acid, B methanol	
Injection volume:	2 µL of 10 ppm mixture in 10% methanol	
Flow rate:	0.6 mL/min	
Gradient:	Time (min)	% B
	0	40
	1	40
	5	80
	8	80
Temperature:	40 °C	
Detector:	UV, 280 nm	

Method development on Poroshell 120 SB-Aq

The method should be modified according to the above description on Poroshell 120 SB-Aq with acetonitrile:1.5% acetic acid mobile phase, because the real samples (butter) are more complex than the standards. For good separation when dealing with interference components in butter, the gradient was adjusted to get ideal resolutions of target antioxidants. Figure 3 shows the chromatogram of the nine antioxidants separated under the modified gradient at 10 ppm. All the compounds had a symmetrical peak shape, and the eluted time of the last peak was extended to 8.5 minutes from 5.2 minutes under the original gradient. It was necessary for the real sample separation to use a slightly longer gradient to get ideal separations.

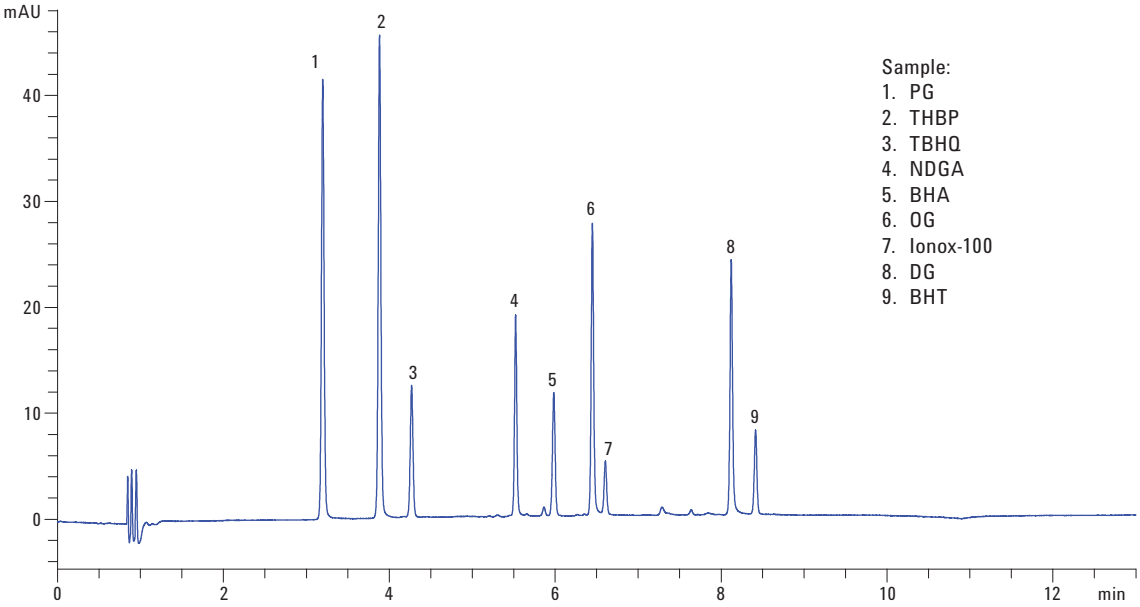


Figure 3. Chromatogram of 10 ppm standards' separation with an Agilent Poroshell 120 SB-Aq column.

Conditions, Figure 3

Eluent:	A 1.5% acetic acid, B ACN	
Injection volume:	2 µL of 10 ppm mixture in 50% ACN/50% IPA	
Flow rate:	0.6 mL/min	
Gradient:	Time (min)	% B
	0	10
	9	75
	10	95
Stop time:	15 minutes	
Temperature:	40 °C	
Detector:	UV, 280 nm	

The data of correlation of linearity and limit of detection (LOD) by UV at 280 nm is shown in Table 2. The coefficients of linearity were excellent for all nine compounds. The LODs were calculated with a signal-to-noise ratio of 3. The LOD was equal to or below 0.1 ppm for all the antioxidants. This corresponded to approximately 0.2 mg/kg or below in a butter sample.

Stable retention times are important for correct identification of analytes in complex food matrixes. Figure 4 shows overlay chromatograms of eight consecutive injections of 1 ppm standard mixture. The retention time of all the peaks was well reproducible and all the relative standard deviations of peak retention time from eight replicate injections on Poroshell 120 SB-Aq column were less than 0.1%.

Table 2. Correlation of linearity and theoretical LOD of nine antioxidants.

No.	Name	Calibration curve	Range (ppm)	Correlation	LOD (s/n=3) (ppm)
1	PG	Y = 9.144X +0.936	0.2 ~ 10	0.9997	0.046
2	THBP	Y = 10.075X + 1.028	0.2 ~ 10	0.9998	0.039
3	TBHQ	Y = 2.815X + 0.309	0.2 ~ 10	0.9997	0.074
4	NDGA	Y = 3.659X +0.306	0.2 ~ 10	0.9997	0.111
5	BHA	Y = 2.631 X +0.330	0.2 ~ 10	0.9997	0.082
6	OG	Y = 5.796 X +0.550	0.2 ~ 10	0.9997	0.037
7	Ionox-100	Y = 1.132 X + 0.130	0.2 ~ 10	0.9994	0.200
8	DG	Y = 5.208X + 0.112	0.2 ~ 10	0.9995	0.090
9	BHT	Y = 1.709 X +0.463	0.2 ~ 10	0.9992	0.118

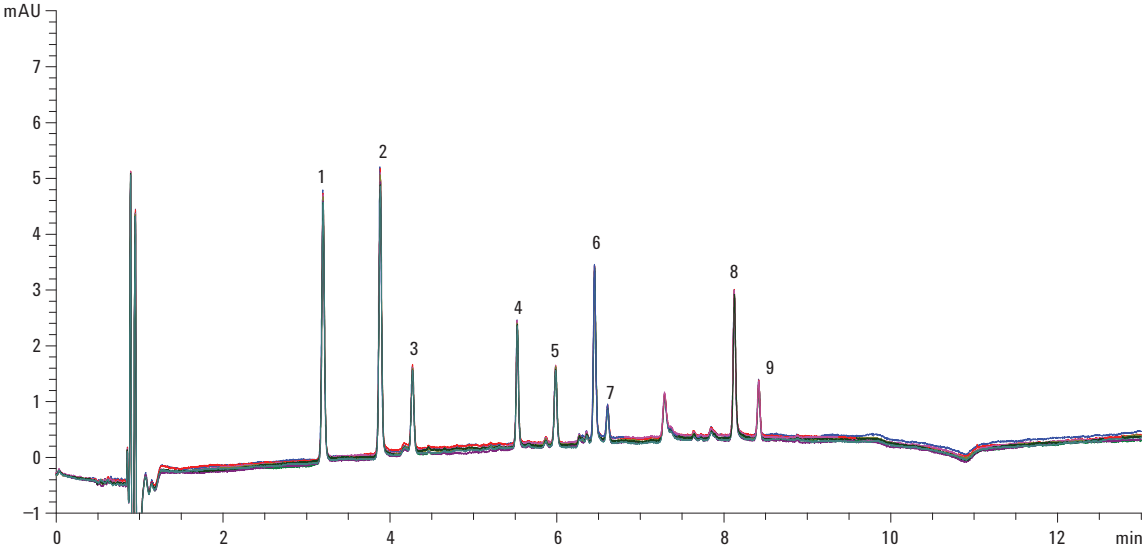


Figure 4. Overlay of chromatograms of eight injections of 1 ppm standards with an Agilent Poroshell 120 SB-Aq column. Conditions same as for Figure 3.

Extracts of five different kinds of butters (Figure 5) and 1 ppm spiked butters (Figure 6) were analyzed to investigate system suitability. Comparing the chromatograms of samples and 1 ppm spiked samples, additional peaks originating from the butter matrix were visible, but there were only a few interferences with the standard peaks. For example, peak 3 was difficult to differentiate from unspiked sample 1 and sample 5, while peak 9 was difficult to differentiate from unspiked sample 2. Though some interferences were found, we can still measure the amounts of antioxidants from the butter at the ppm level using the HPLC method. If a lower level of LOD is needed, a triple-quadrupole mass spectrometer would be a better choice of detector.

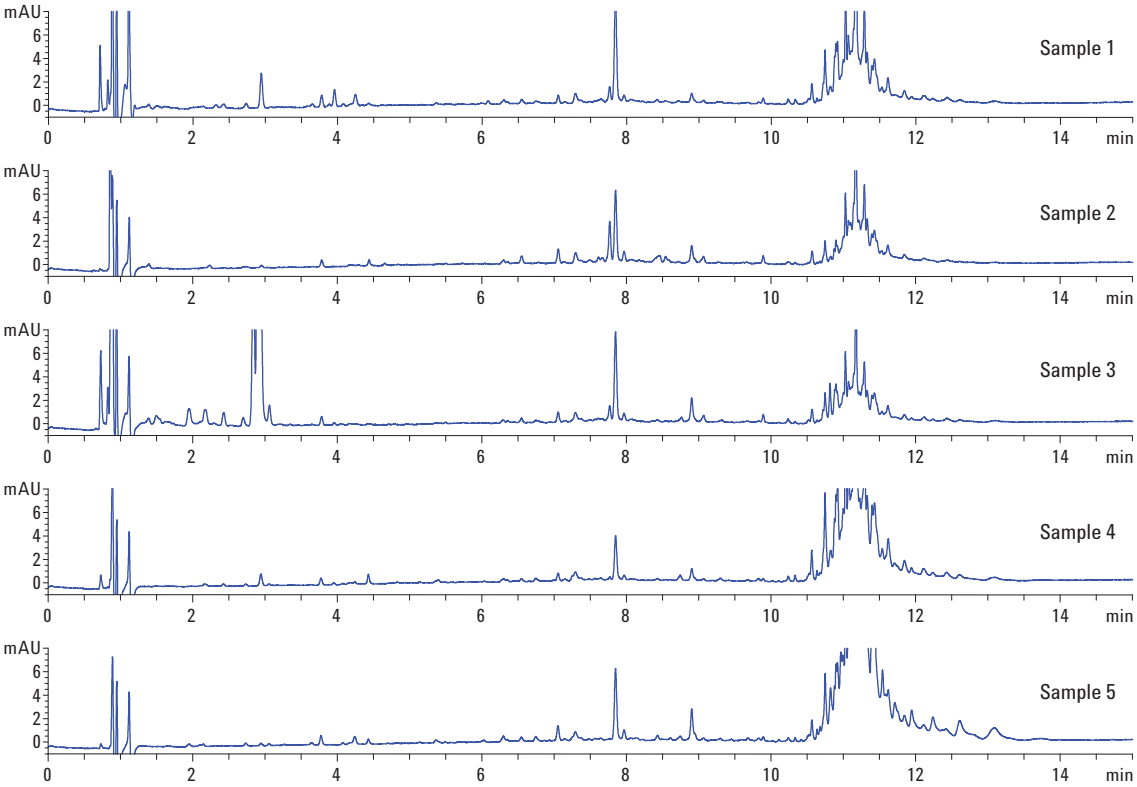


Figure 5. Separation of five different kinds of butter with an Agilent Poroshell 120 SB-Aq column. Conditions same as for Figure 3.

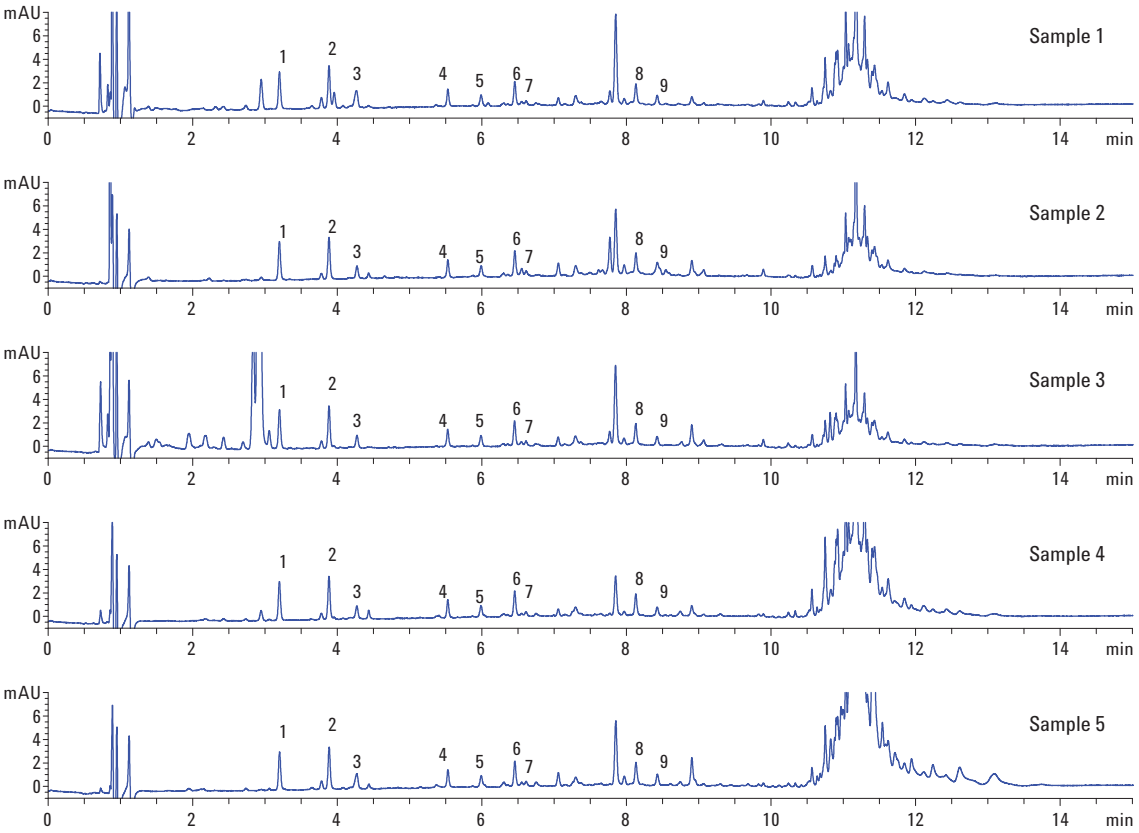


Figure 6. Separation of five different kinds of butter spiked with 1 ppm standards mixture using an Agilent Poroshell 120 SB-Aq column. Conditions same as for Figure 3.

Conclusions

Agilent Poroshell 120 columns are packed with superficially porous particles, which provide high performance similar to the sub-2 µm particles but with a 40 to 50% lower pressure than columns with sub-2 µm particles. The recent introduction of new bonded phases on Poroshell 120 makes them useful in method development by offering unique and differing selectivities across the various chemistries.

Using Poroshell 120 SB-Aq with the Agilent 1290 Infinity LC, a quick analytical method was developed for the determination of antioxidants in butters. The analysis time could be reduced to 15 minutes, one-third of the analysis time with a traditional 5 µm column. Butter samples and spiked butter samples were extracted and the system suitability was investigated. Satisfactory results were achieved at a ppm level. The developed method was suitable for QC laboratories in the food industry for the antioxidant analysis of butter.

References

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