Determination of preservatives in food and drugstore products with the Agilent 1120 Compact LC

Application Note

Food

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Abstract

This Application Note describes the analysis of several well known preservatives like benzoic acid, sorbic acid or the esters p-hydroxy benzoic acid (methyl-, ethyl- and propyl-) with the Agilent 1120 Compact LC.

Conventional LC methods are often used in routine analyses to characterize or monitor products for this purpose. The presented data show the determination of these ingredients in several consumer products such as chewing gum, ketchup, and barbecue sauce, which demonstrates the relevance of this method for quality control testing and monitoring of products for consumer protection.

This Application Note shows that the Agilent 1120 Compact LC works as a reliable and highly robust instrument for standard LC. It can be used for routine analyses. The use of methanol for separating the components reduces cost and allows a 15-min determination of all components with an overall resolution of >3. The results of reliability, quality, system suitability and performance testing are shown.

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Introduction

Several consumer products contain preservatives, which can be harmful to some people. The most common preservatives are benzoic or sorbic acid as well as the esters p-hydroxy benzoic acid. These ingredients inhibit microbiological growth in food or drugstore products and are used either alone or as a mixture. During manufacturing, the products are analyzed for quality control and monitored for consumer protection. Standardized conventional LC methods are often used in routine analyses to characterize or monitor products.

There are several requirements for the analytical instrumentation: reliability, flexibility, and ease of use. In addition, the instrument should provide low ownership cost.

This Application Note shows that the Agilent 1120 Compact LC works as a reliable and highly robust instrument for standard LC. It can be used in routine analyses, for preservatives found in several consumer products. These analyses are done in independent control laboratories; therefore the use of methanol for separating the components should be optimized to reduce cost and pollution.

The results of reliability, quality, system suitability and performance tests are shown.

Instrumentation

An Agilent 1120 Compact LC with the following configuration was used:

Configuration of the Agilent 1120 Compact LC
Gradient pump and vacuum degasser
Auto sampler
Column oven
Variable wavelength detector
Software: EZ-Chrom Elite Compact 3.3

Preparation of samples

Reference samples

Dissolve 10 mg of each vitamin in water and dilute to 100 mL with the same solvent. Mix and dilute 1 mL of each solution to 20 mL with mobile phase.

The following common preservatives were checked:
Benzoic acid, sorbic acid, methyl-, ethyl- and propylesters of p-hydroxy benzoic acid (Methyl-PHB, Ethyl-PHB, Propyl-PHB).

Samples from food and drugstores

Dissolve 1g of sample in 10 mL of water. Solution and extraction can be improved by treating for 10 min in a ultrasonic bath. Next, filter the samples with syringe filters; first through a 2-µm filter, followed by a 0.45-µm syringe filter to prepare the clear solution for injection.

Chromatographic conditions		
Column	ZORBAX Eclipse XDB C18, 150 mm × 4.6 mm, 5 µm	
Mobile phases		
Phase A:	Dissolve 6.8 g potassium dihydrogen phosphate in 900 mL water. The pH value should be adjusted to pH = 2.3 with phosphoric acid and then filled to 1000 mL with water.	
Phase B:	Methanol	
Gradient (linear):	Time (min)	
	0	80% A/20% B
	9.6	47% A/53% B
	12	33% A/67% B
	13	33% A/67% B
	13.1	80% A/20% B
Pump settings		
Stop time:	15 min	
Post time:	5 min	
Flow rate:	1.75 mL/min	
Autosampler		
Injection volume:	20 µL	
Thermostatted column compartment		
Temperature: 40	°C	
Detector	14 µL cell, Peak width: >0.05 min, 1 s response time (10 Hz), Signal: 220 nm	

System suitability and performance test:

For system suitability testing, the reference solution with the limits listed below was used. This was in accordance with Q3A(R)- Impurities in New Drug Substances:[2]

- Resolution: minimum 1.5 between each peak
- Precision of areas must be < 2 % RSD.
- Precision of retention times must be < 0.5 % RSD.

With these limits and settings for testing, the samples in Table 1 were prepared and analyzed.

Sample	Purpose	Number of injections
Blanc solution	Verify baseline stability and identify artifacts	2
Calibration samples	Verify linearity	3 of each level
Control sample	Verify sensitivity and resolution for reference solution	6
Suitability sample	Verify precision of areas and retention times for reference solution	10

Table 1
Setup for testing.

Results and discussion

The separation was achieved with methanol, since it was able to separate the critical pair of benzoic and sorbic acid.[2] The results in Figure 1 show good separation of all preservatives with the Agilent ZORBAX Eclipse XDB C18 material on the Agilent 1120 Compact LC and EZChrom Elite Compact Software.

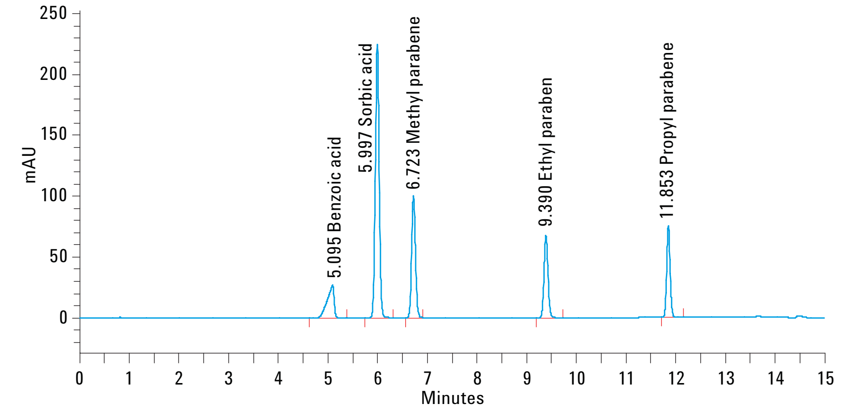

Figure 1
Standard chromatogram of preservatives with the Agilent 1120 Compact LC.

For the detection of all components, the Agilent 1120 Compact LC variable wavelength detector was set for 260 nm. This setting was chosen because all target analytes except benzoic acid has a maximum adsorption near 260 nm.

Detailed data are listed in Table 2. The first limit for resolution (>1.5) is fulfilled for all peaks. The data for resolution show good selectivity with the ZORBAX Eclipse XDB C18 material.

Compound	Retention time (min)	Resolution
Benzoic acid	5.06	–
Ascorbic acid	5.96	4.31
Methyl-PHB	6.69	5.25
Ethyl-PHB	9.39	18.55
Propyl-PHB	11.85	17.83

Table 2
Results for control sample: Retention times and resolution.

The areas and retention time precision results of all compounds of the suitability sample are shown in Table 3. The data demonstrate the high reliability and precision of the Agilent 1120 Compact LC. The data show that the system can be used for QC methods, since the criteria for retention times and areas are fulfilled for all compounds.

The data in Tables 3 and 4 prove high precision and reliability of the autosampler. The correlation coefficient for each calibration curve is very close to 1.0 showing high versatility and quality for QC testing and monitoring.

It can be seen that with the selected column, as well as with the Agilent 1120 Compact LC, characterization and monitoring of products from foods and drugs is possible. The following examples demonstrate the performance of the system:

- The chromatogram of Figure 2 illustrates that the chewing gum is free of all of the preservatives tested.

Compound	Retention time (min)	RSD RT n = 10	RSD Area n = 10	Asymmetry n = 10
Benzoic acid	5.06	0.408	0.188	0.64
Ascorbic acid	5.96	0.153	0.235	1.00
Methyl-PHB	6.69	0.277	0.260	1.09
Ethyl-PHB	9.39	0.039	0.112	1.09
Propyl-PHB	11.85	0.009	0.074	1.10

Table 3
Suitability sample: Precision of retention times and areas for the Agilent 1120 Compact LC.

Compound	m	b	r
Benzoic acid	2151314.8	25187.3	0.999
Ascorbic acid	10188005.0	119381.1	1.0
Methyl-PHB	4459268.1	83135.1	1.0
Ethyl-PHB	3097910.6	57372.8	1.0
Propyl-PHB	3024691.2	24203.6	1.0

Table 4
Calibration data of the Agilent 1120 Comapct LC (Setting "Ignore Origin", Y = mx + b, 1.0 µg/mL–20.0 µg/mL.)

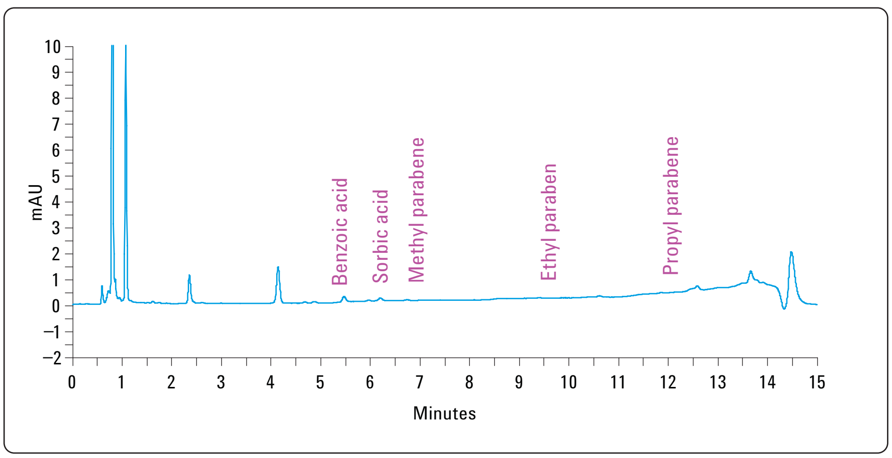

Figure 2
Analysis of preservative "free" chewing gum.

- The chromatogram of Figure 3 shows that the mouthwash tested is free of preservatives tested, but it is possible that different preservatives were used to inhibit microbial growth.
- The chromatograms in Figures 4 and 5 show the analysis of a beauty cream and a toothpaste. The declared preservative (Methyl-PHB) is part of the product. This chromatogram verifies that the method can be used for quality control as well as for monitoring.

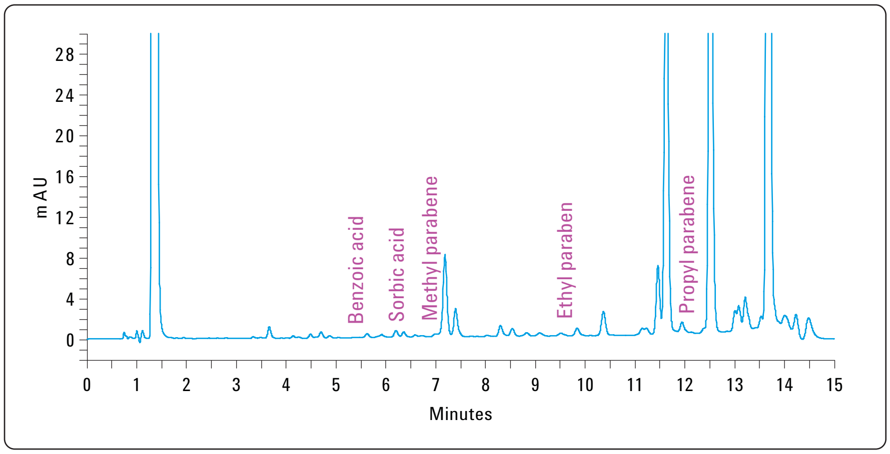

Figure 3
Analysis of mouthwash.

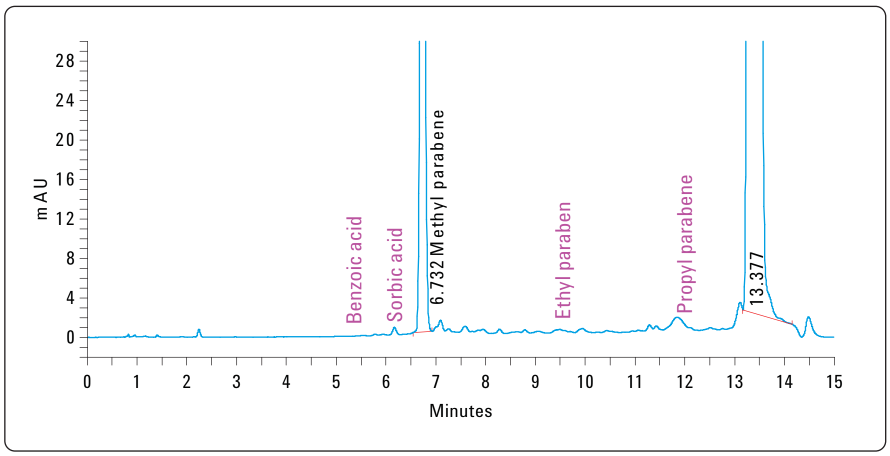

Figure 4
Chromatogram of a beauty cream.

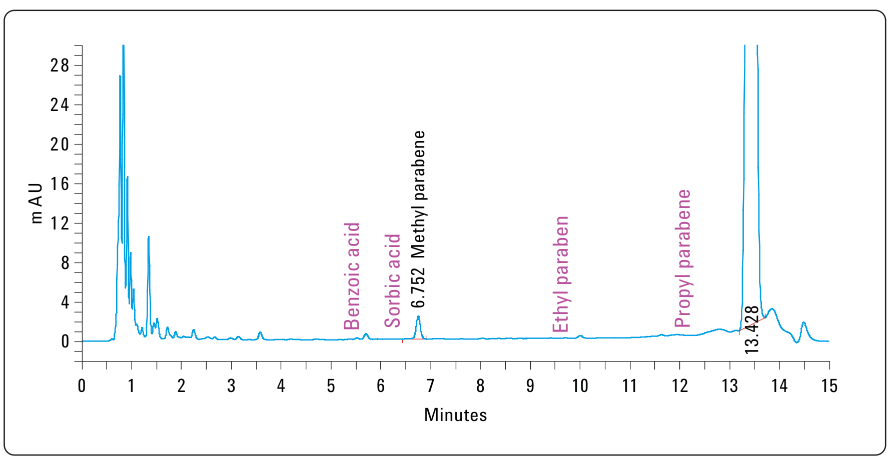

Figure 5
Chromatogram of a toothpaste.

• The chromatograms in Figures 6 and 7 show the method can monitor supermarket products. The preservative-free brand of ketchup is proven to be free of the tested preservatives. The preservative-free barbecue sauce contains some considerable amounts of benzoic acid and methyl-PHB.

All examples show minimal influence of the matrix on the separation. It can be seen that it is easy to detect any of the tested preservatives.

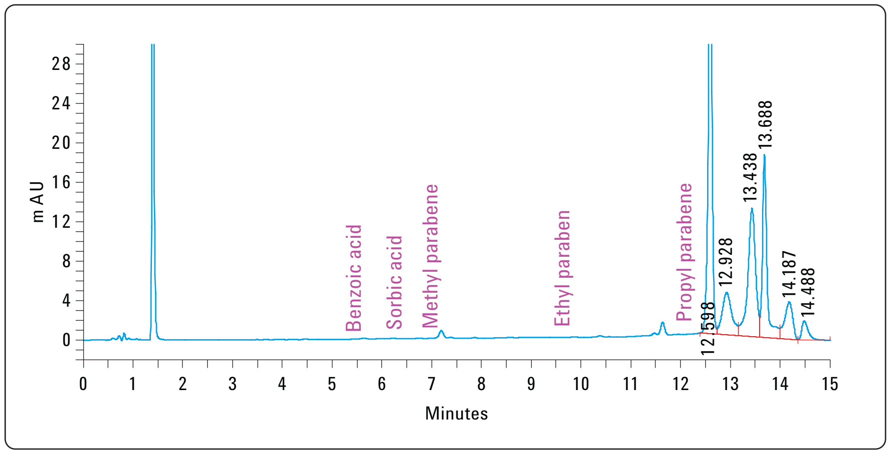

Figure 6
Analysis of a "preservative free" ketchup brand.

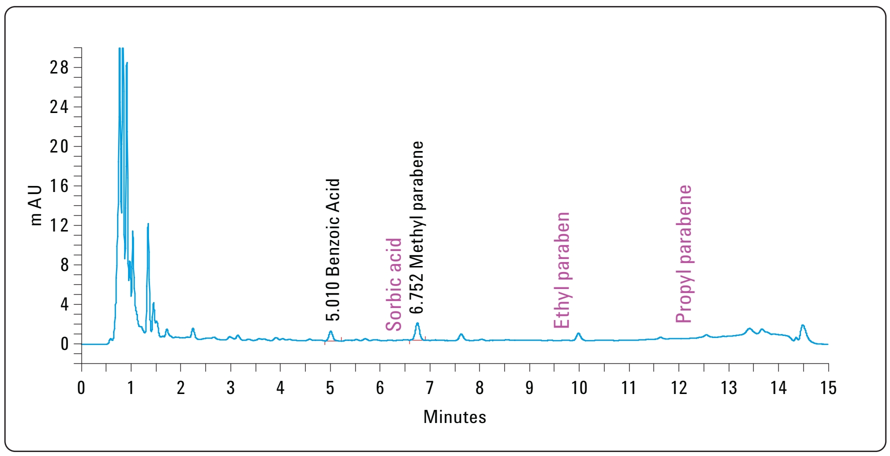

Figure 7
Analysis of a "preservative free" barbecue sauce.

Conclusion

The Agilent 1120 Compact LC is designed for users in independent labs who require LC methodology with reliability, ease-of-use, and low ownership cost, to characterize or monitor products.

This Application Note shows a reliable approach for the determination of preservatives in food or drugstore products. The data prove high precision of retention time, and provide clear chromatographic parameters such as resolution and asymmetry.

As shown in Table 2, the resolution of all main peaks was found to be greater than 3.0. The calibration data of each compound shows that the instrument can be operated as required in a quality control environment. All criteria for precision, such as areas and retention times are fulfilled (see Table 3). The results indicate that the use of the Agilent 1120 Compact LC in QA/QC laboratories to characterize products containing preservatives is appropriate. In addition, it is possible to identify any of the tested preservatives independent of the matrix.

All results explicitly show the applicability of the Agilent 1120 Compact LC for quality control testing with reduced costs per system and improved simplicity of use. In addition to the instrument capabilities, the new version of the EZChrom Elite compact software allows full control of the Agilent 1120 Compact LC with a wide range of features for data analysis and results reporting.

The results for resolution and asymmetry show good selectivity and performance with the ZORBAX Eclipse XDB.

The Agilent 1120 Compact LC is qualified and optimized for everyday productivity and routine analysis.

References

1. "Analysis of Preservatives in White Wine an Salad Dressing using HPLC," 1997, Agilent Technologies publication 5966-0629E

2. Q3A(R) Impurities in New Drug Substances, Rev. 2, http://www.fda.gov/cder/guidance/7838fnl.pdf,

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