

# The Agilent 1290 Infinity II Multi-Method Solution

Switch between seven applications on a single  
LC system by using automatic column and solvent  
selection

Suitable for Agilent  
1290 Infinity III LC

## Application Note

Food Testing & Agriculture

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### Abstract

This Application Note describes the use of the Agilent 1290 Infinity II LC for the automated operation of seven different applications using different methods (different stationary and mobile phases) in food analysis. The exchange of columns is controlled by the method used. All columns are located in a single Agilent 1290 Infinity II Multicolumn Thermostat. Accordingly, the Agilent 1290 Infinity II Flexible Pump is attached to two external solvent-selection valves for automated solvent switching, according to the method.



**Agilent Technologies**

## Introduction

A typical laboratory that analyzes food quality offers a large variety of measurements for different applications, for example, mycotoxins, parabens, and so on. These applications are usually measured on one UHPLC instrument, making it necessary to exchange columns and solvents to adapt the instrument to the desired application. If these adaptations are done manually, analyzing a larger variety of analytes is time-consuming. If an application finishes after the working day, the instrument waits for hours or even days for manual interaction and a restart. This can be overcome by using an instrument that employs multiple columns with multiple solvents, such as the Agilent 1290 Infinity II LC. This system can operate up to eight columns with up to 26 different solvents, enabling more than 1,000 different analytical conditions. The individual combination of solvent and column is chosen by a predefined method optimized for the analysis of the desired analytes, making it easy to automatically change the analysis conditions within a sequence for different analyses.

The system we describe comprises two highly sophisticated modules from the Agilent 1290 Infinity II LC, the Agilent 1290 Infinity II Multicolumn Thermostat (MCT) and the Agilent 1290 Infinity II Multisampler, which enhance the system so that it delivers the highest possible performance.

The 1290 Infinity II MCT enables precise column thermostating over a broad temperature range. Heat exchange is done by heat exchangers with the lowest dead volume and highest efficiency, which enables excellent retention time stability for highly reproducible retention times<sup>1</sup>.

The 1290 Infinity II Multisampler, with its advanced design elements such as sample hotel, needle handling routines, and carryover reduction, handles a large number of different samples and analyte types without interactions<sup>2</sup>. This allows optimum performance for a multimethod analysis system.

We describe the use of the 1290 Infinity II LC with an eight-column selection valve located in the 1290 Infinity II MCT, and two solvent-selection valves attached to the 1290 Infinity II Flexible Pump for multimethod analysis.

## Experimental

### Instrumentation

The Agilent 1290 Infinity II LC solution for multimethod analysis comprises:

- Agilent 1290 Infinity II Flexible Pump (G7104A)
- Agilent 1290 Infinity II Multisampler (G7167B)

- Agilent 1290 Infinity II Multicolumn Thermostat MCT (G7116B) with valve drive (G7116B#058) equipped with Agilent Quick-Change eight-column selector valve (G4239C) and capillary kit (G4239C#005, p/n 5067-4248). This kit contains all Agilent Quick-Connect heat exchangers (standard flow) and capillaries needed for the installation of up to eight columns.
- Agilent 1290 Infinity II DAD (G7117B)
- Agilent 1290 Infinity External Valve Drive (2 x G1170A) with Quick-Change solvent selector valve (2 x G4235A)

### Software

Agilent OpenLAB A.02.02 CDS, ChemStation Edition for LC and LC/MS Systems, Rev. C.01.07

### Instrument setup

The columns used for all applications were introduced in the column list in ChemStation (Figure 1). In the ChemStation column list, open a new line by clicking **Append** to add a new column. For the columns, a description, geometric data, particle size, and limitations such as pH, pressure, and temperature must be given. Installed analytical columns are indicated in the table by YES.

Edit Columns: Infinity II MethDev

Insert

Append

Delete

Print

OK

Cancel

Help

#	Installed	Description	Col. Serial#	Batch#	Product#	# Injections	Max. p [bar]	Max. T [°C]	Max. pH	Min. pH	Length	Diameter	Size	Void	Unit	Comment
1	YES	EclipsePlus-C8 2.1	autoID-12		959764-906	0	600	60.0	9.0	2.0	100.0	2.1	1.8	60.00	%	
2	YES	Poroshell 120EC-C	autoID-13		695975-302	0	600	60.0	9.0	2.0	100.0	3.0	2.7	60.00	%	
3	YES	SB-C18 2, 1x50mm	autoID-14		827700-902	0	600	60.0	9.0	2.0	50.0	2.1	1.8	60.00	%	
4	YES	Eclipse Plus C18 2	autoID-15		959758-902	0	600	60.0	9.0	2.0	50.0	2.1	1.8	60.00	%	
5	YES	SB-C8	autoID-16		828700-906	0	600	60.0	9.0	2.0	100.0	2.1	1.8	60.00	%	
6	YES	Extend-C18	autoID-17		728700-902	0	600	60.0	10.0	2.0	100.0	2.1	1.8	60.00	%	
7	YES	Eclipse Plus C18	autoID-18		959741-902	0	600	60.0	9.0	2.0	50.0	2.1	1.8	60.00	%	
8	no	Eclipse XDB-C18	autoID-6		927975-902	0	600	60.0	9.0	2.0	50.0	4.6	1.8	60.00	%	
9	no	SB-C18	autoID-7		827700-902	0	600	90.0	8.0	1.0	50.0	2.1	1.8	60.00	%	
10	no	SB-C18	autoID-8		827975-302	0	600	90.0	8.0	1.0	50.0	3.0	1.8	60.00	%	
11	no	SB-C18	autoID-9		827975-902	0	600	90.0	8.0	1.0	50.0	4.6	1.8	60.00	%	
12	no	Eclipse Plus C18	autoID-10		959941-902	0	600	60.0	9.0	2.0	50.0	4.6	1.8	60.00	%	
13	no	Eclipse XDB-C18	autoID-11		993967-902	0	400	60.0	9.0	2.0	150.0	4.6	5.0	60.00	%	

Figure 1. The Agilent ChemStation column list that provides an overview of all columns available in the laboratory.

The column list is directly connected to the column assignment in the 1290 Infinity II MCT (Figure 2). In the assignment, the position of the column on the right or left can be chosen and associated with a color code. The column data from the column list can be selected under Column Tag Information and associated to a column position and color code.

The column used for the individual method can be selected in the Method screen of the 1290 Infinity II MCT (Figure 3). The appropriate column is chosen either by the pull-down menu, which shows all assigned columns, or by just clicking the column with the right color code in the image of the 1290 Infinity II MCT. The current valve position, which then connects automatically to the chosen column, is shown. For quick information, the valve position, the color code of the chosen column, and its product number are shown in the ChemStation user interface (Figure 4).

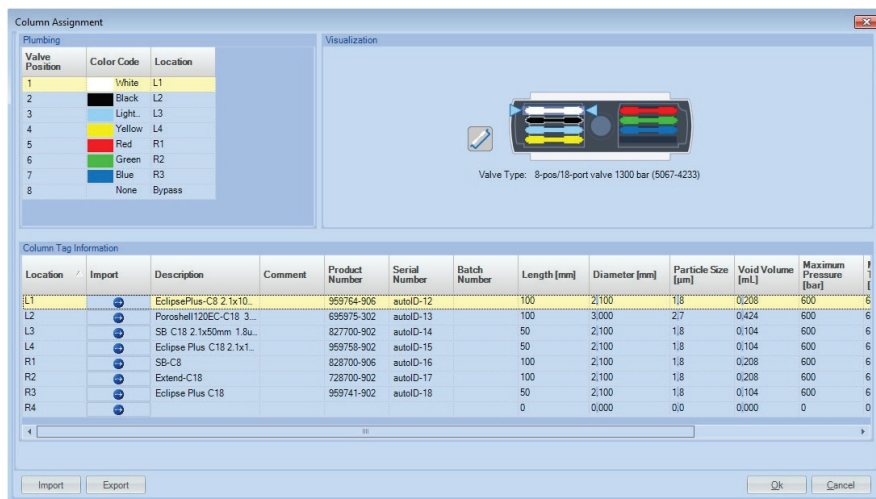


Figure 2. Column assignment in the Agilent 1290 Infinity II MCT. Each column is assigned to an unambiguous location in the MCT.

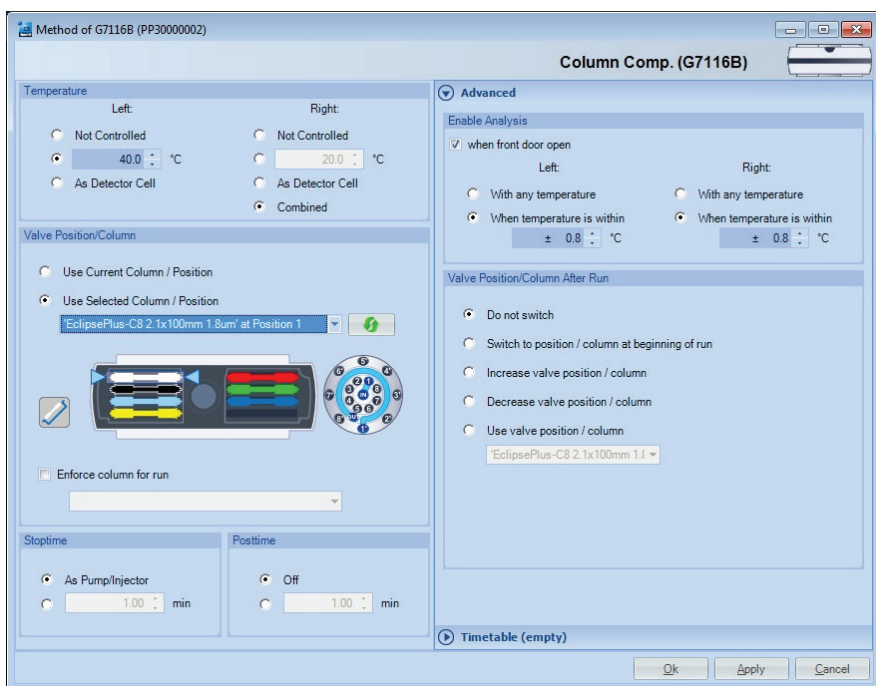


Figure 3. Column selection in the Method screen of the Agilent 1290 Infinity II MCT.

The assignment of the solvents is done in a similar way. The solvent selection valves are assigned to the pump channels in the instrument configuration (not shown). In the Pump Valve Cluster Configuration screen, the positions of the solvent selection valve are named by the connected solvent (Figure 5). The compressibility calibration is chosen by taking the correct solvent or type of solvent from the pull-down menu. Molarity and pH values can be added. In the Method of Pump Valve Cluster screen, the right solvents for channels A and B are chosen and the valve automatically switches to the correct positions (Figure 6). The accessible multiple solvents are sketched out in the ChemStation pump user interface (Figure 4).

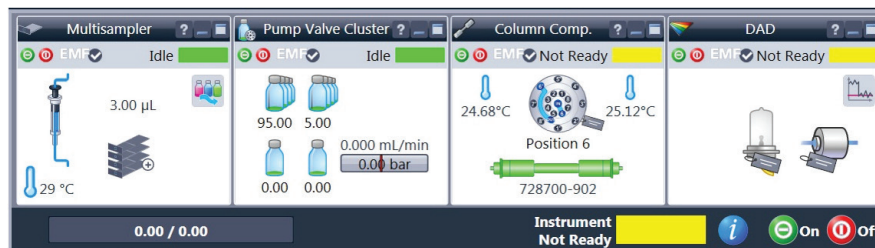


Figure 4. Instrument user interface in Agilent ChemStation, highlighting the active column.

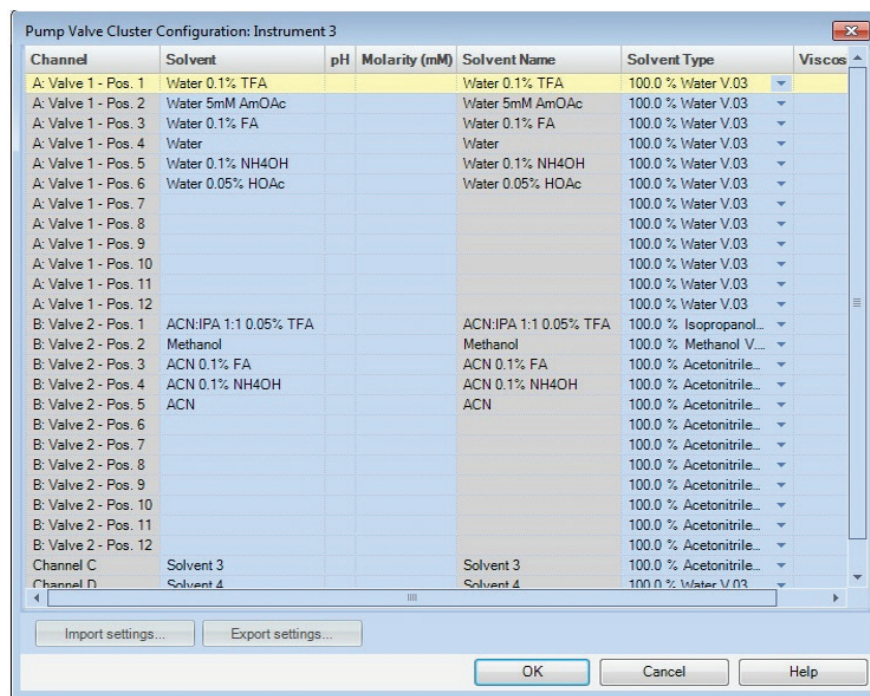


Figure 5. The Pump Valve Cluster Configuration screen.

## Methods

The detailed methods for the analysis of the individual compounds are in Results and Discussion. The main analysis conditions for each group of analytes are summarized in Table 1.

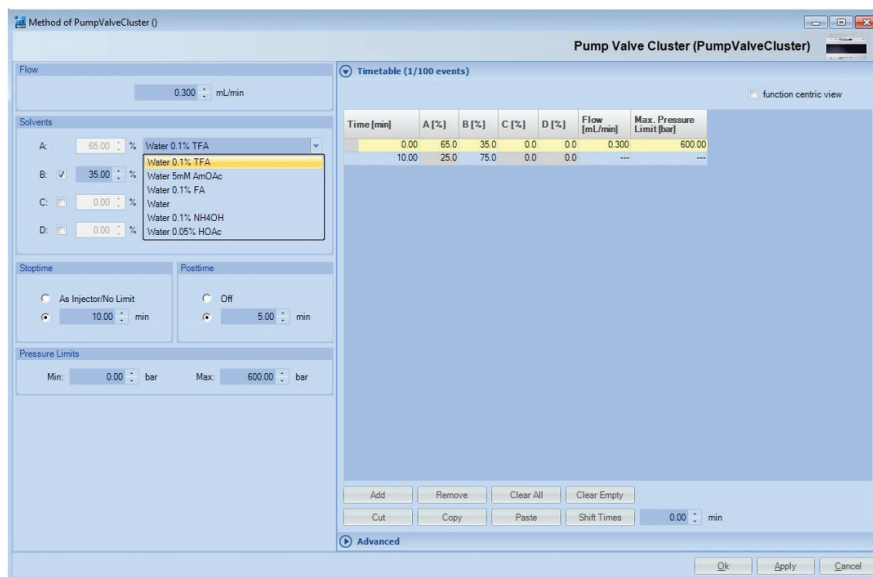


Figure 6. The Method of Pump Valve Cluster screen.

Table 1. Summary of main analytical conditions for each group of analytes.

Compound	Column	Mobile phase A	Mobile phase B	Temperature
<b>Preservatives</b> Benzoic acid Salicylic acid Citric acid	Agilent ZORBAX RRHD Eclipse C8, 2.1 × 100 mm, 1.8 µm (p/n 959764-906)	Water + 0.05% TFA pH 2.1	Acetonitrile:isopropanol (1:1) + 0.05% TFA	40 °C
<b>Parabens</b> Methyl paraben Ethyl paraben Propyl paraben Butyl paraben	Agilent Poroshell 120 C18, 3.0 × 100 mm, 1.8 µm (p/n 695975-302)	Water + 5 mM NH <sub>4</sub> OAc pH 6.73	Methanol	45 °C
<b>Antiparasitic</b> Malachite green	Agilent ZORBAX RRHD SB C18, 2.1 × 50 mm, 1.8 µm (p/n 827700-902)	Water + 0.1% formic acid pH 2.7	Acetonitrile + 0.1% formic acid	40 °C
<b>Coloring additives</b> Sudan Red I Sudan Red II Sudan Red III Sudan Red IV	Agilent ZORBAX RRHD Eclipse plus C18, 2.1 × 100 mm, 1.8 µm (p/n 959758-902)	Water + 0.1% formic acid pH 2.7	Acetonitrile + 0.1% formic acid	30 °C
<b>Antimicrobial</b> Chloramphenicol	Agilent ZORBAX RRHD SB C8, 2.1 × 100 mm, 1.8 µm (p/n 828700-906)	Water	Methanol	30 °C
<b>Mycotoxin</b> Patulin	Agilent ZORBAX RRHD Extend C18, 2.1 × 100 mm, 1.8 µm (p/n 728700-902)	Water + 0.1% NH <sub>4</sub> OH pH 10.3	Acetonitrile + 0.1% NH <sub>4</sub> OH	40 °C
<b>Antioxidants</b> PG TBHQ BHA BHT	Agilent ZORBAX RRHD Eclipse C18, 2.1 × 50 mm, 1.8 µm (p/n 959741-902)	Water + 0.05% acetic acid pH 3.43	Acetonitrile	40 °C



After an exchange of a method in a sequence of samples, which includes the exchange of the column and the solvents, it is necessary to flush the system with the new solvents and equilibrate the column on the starting conditions of the method.

### Generation of solvent exchange methods

The exchange of the solvent can be done by taking the analytical method for the desired application. The solvent composition was set to 50:50 and the column selection valve switched to the bypass position (a capillary was installed on position eight instead of a column). Under these conditions, the solvents were run through the system at 5 mL/min for 4 minutes isocratically and without injection. The created method can be stored separately and used in sequence.

### Generation of column equilibration methods

For the equilibration of the column, the analytical method was taken and run without injection under isocratic conditions at the starting solvent composition. The run time was the same as used for re-equilibration between sample runs. The created method can be stored separately and used in sequence.

### Chemicals

All chemicals were purchased from Sigma-Aldrich, Corp., Germany. All solvents were purchased from Merck, Germany. Fresh ultrapure water was obtained from a Milli-Q Integral system equipped with LC-Pak Polisher and a 0.22- $\mu$ m membrane point-of-use cartridge (Millipak).

## Results and Discussion

The following analyses can be done in a single sequence by exchanging the column and the solvents with the method. After each exchange of solvents, a solvent wash method must be run. In addition, after an exchange of the column, the chosen column must be conditioned using the following starting conditions with the respective method.

### Analysis of preservatives

Preservatives are used to prevent the growth of fungi and bacteria in food and beverages. Typical compounds are sodium benzoate (E211), salicylic acid, and sorbic acid (E200).

Parameter	Value
Test sample	Sorbic acid, benzoic acid, salicylic acid, 30 mg/100 mL methanol each, 1:10 dilution
Gradient	5 to 50% B in 10 minutes
Stop time	10 minutes
Post time	5 minutes
Flow rate	0.4 mL/min
Injection volume	1 $\mu$ L
Wash	6 seconds with methanol
Column temperature	45 $^{\circ}$ C
DAD	205/4 nm, Ref. 360/100 nm, data rate 10 Hz, path length 10 mm, slit 8 nm

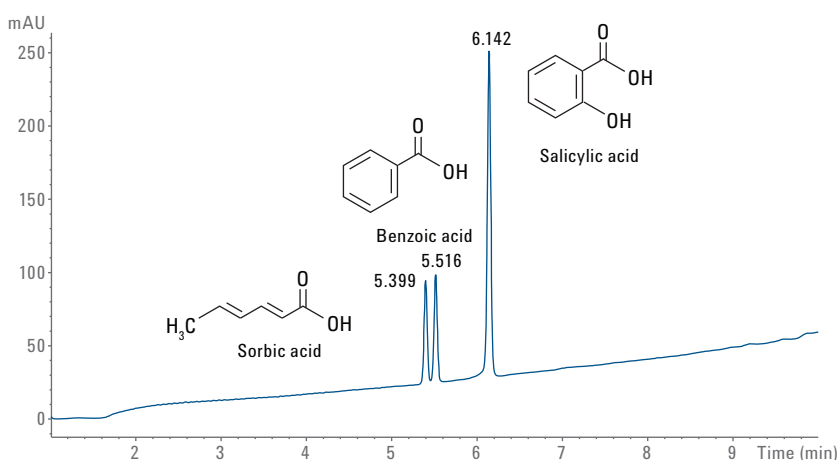


Figure 7. Separation of the food preservatives sorbic acid, benzoic acid, and salicylic acid.

The analysis methods and results belong to preservatives, parabens, antiparasitic drugs, coloring additives, antimicrobial drugs, mycotoxins, and antioxidants (Figures 7 to 13). These compounds can be found in food and beverages.

## Parabens

Parabens are typically used as preservatives in cosmetics and pharmaceuticals. Widely used parabens are methyl paraben (E218), ethyl paraben (E214), propyl paraben (E216), and butyl paraben.

Parameter	Value
Test sample	Methyl, ethyl, propyl, butyl paraben, 10 mg each, dissolved in 100 mL acetonitrile, diluted 1:10 with acetonitrile
Gradient	40 to 55% B in 10 minutes
Stop time	10 minutes
Post time	5 minutes
Flow rate	0.5 mL/min
Injection volume	1 µL
Wash	6 seconds with methanol
Column temperature	45 °C
DAD	254/4 nm, Ref. 360/100 nm, data rate 10 Hz, path length 10 mm, slit 8 nm

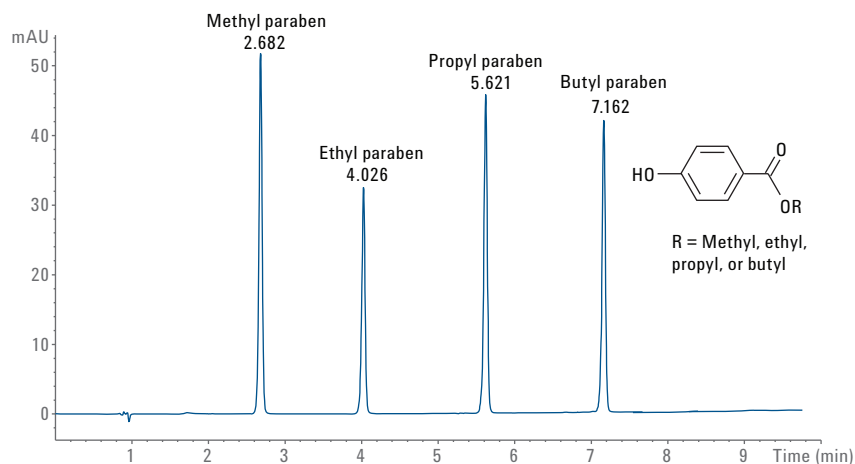


Figure 8. Separation of the preservatives methyl, ethyl, propyl, and butyl paraben, widely used in cosmetics and pharmaceuticals.

## Antiparasitic drugs

Malachite green is a typical antiseptic or antiparasitic drug used against fungal and bacterial infections, especially in fish and fish eggs. It is banned from use in breeding fish for human consumption because of health concerns.

Parameter	Value
Test sample	Malachite green, 2 mg, dissolved in methanol, 10 mL, diluted 1:10 with methanol
Gradient	5 to 95% B in 5 minutes, 2 minutes at 95% B
Stop time	7 minutes
Post time	3 minutes
Flow rate	0.5 mL/min
Injection volume	1 µL
Wash	6 seconds with methanol
Column temperature	40 °C
DAD	550 nm/10 nm, Ref. off, data rate 20 Hz, path length 10 mm, slit 8 nm

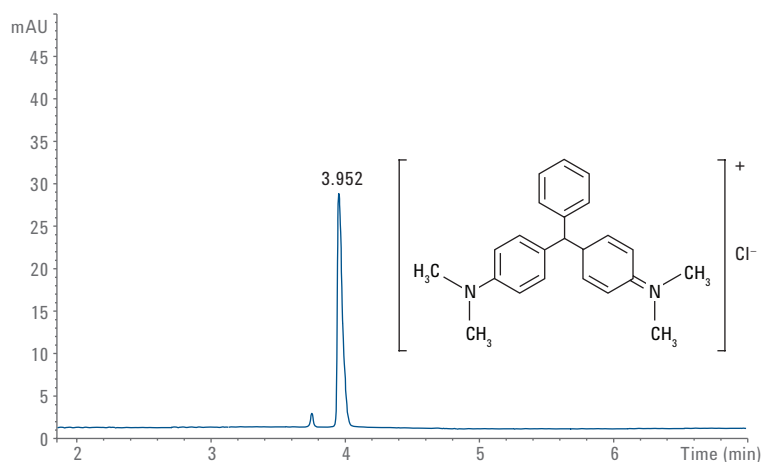


Figure 9. Determination of the antiparasitic drug malachite green, banned from use in fish farming.

## Coloring additives

From the class of azo dyes, the Sudan Red colors have been used to colorize food products such as paprika, curry, and chili powder. However, the use of these Sudan Red colors is now banned as use as food colorizers. In particular, Sudan Red I, Sudan Red II, and Sudan Red IV are classified carcinogens by the International Agency for Research on Cancer.

Parameter	Value
Test sample	Sudan Red I, Sudan Red II, Sudan Red III, Sudan Red IV, 2 mg each, dissolved in 20 mL methanol, diluted 1:10 in methanol
Gradient	80 to 95% B in 10 minutes
Stop time	10 minutes
Post time	5 minutes
Flow rate	0.4 mL/min
Injection volume	1 µL
Wash	6 seconds with methanol
Column temperature	30 °C
DAD	500 nm/10 nm, Ref. off, data rate 10 Hz, path length 10 mm, slit 8 nm

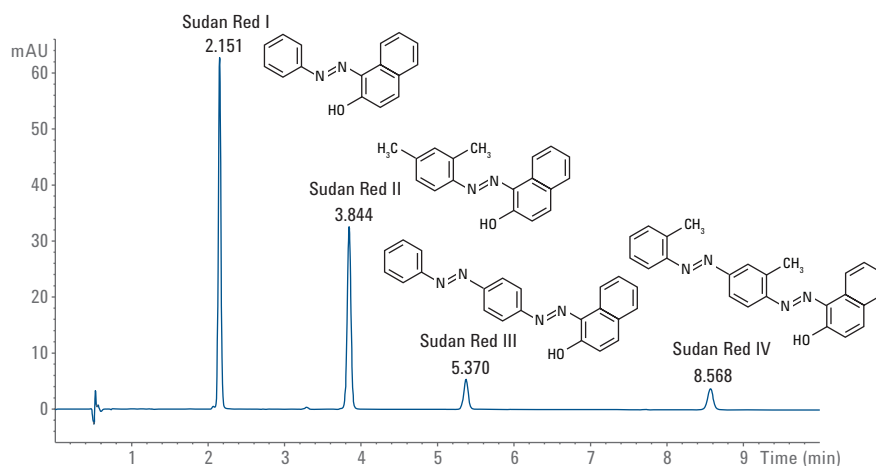


Figure 10. Separation of Sudan Red I, II, III, and IV, formerly used as coloring additives in spices such as chili and paprika, but now banned.

## Antimicrobial drugs

Chloramphenicol is an antibacterial and antimicrobial drug. Due to its broad-spectrum antibiotic behavior, it is used to protect livestock and can be found in food.

Parameter	Value
Test sample	Chloramphenicol, 2 mg, dissolved in 20 mL methanol, diluted 1:10 with methanol
Gradient	10 to 90% B in 10 minutes
Stop time	10 minutes
Post time	5 minutes
Flow rate	0.3 mL/min
Injection volume	1 µL
Wash	6 seconds with methanol
Column temperature	45 °C
DAD	254/4 nm, Ref. 360/100 nm, data rate 10 Hz, path length 10 mm, slit 8 nm

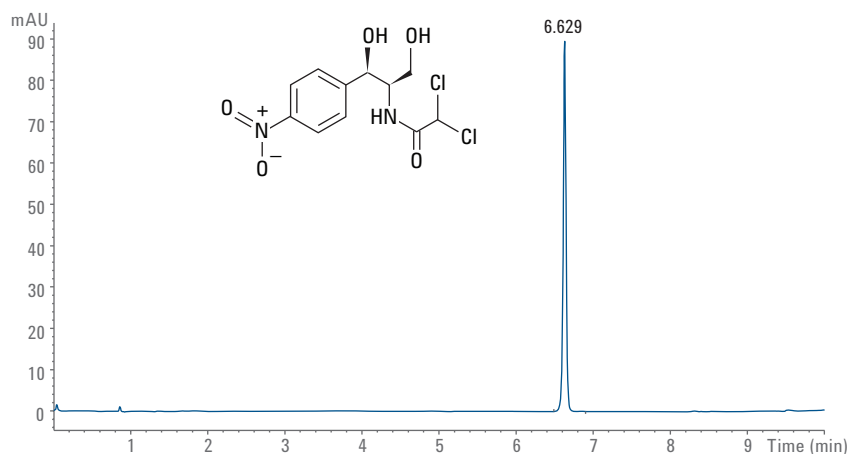


Figure 11. Determination of the broad-spectrum antibiotic chloramphenicol.



## Mycotoxins

The mycotoxin patulin, which is suspected to be genotoxic, is found in rotting apples. Therefore, apple products must be analyzed for this mycotoxin. The World Health Organization recommends limits below maximum concentration of 50 ng/L in, for example, apple juice.

Parameter	Value
Test sample	Patulin, 5 mg, dissolved in 10 mL acetonitrile, diluted 1:20 with acetonitrile
Gradient	5% B for 3 minutes, at 3 minutes to 90% B
Stop time	6 minutes
Post time	3 minutes
Flow rate	0.5 mL/min
Injection volume	1 µL
Wash	6 seconds with methanol
Column temperature	30 °C
DAD	276/4 nm, Ref. 360/100 nm, data rate 10 Hz, path length 10 mm, slit 8 nm

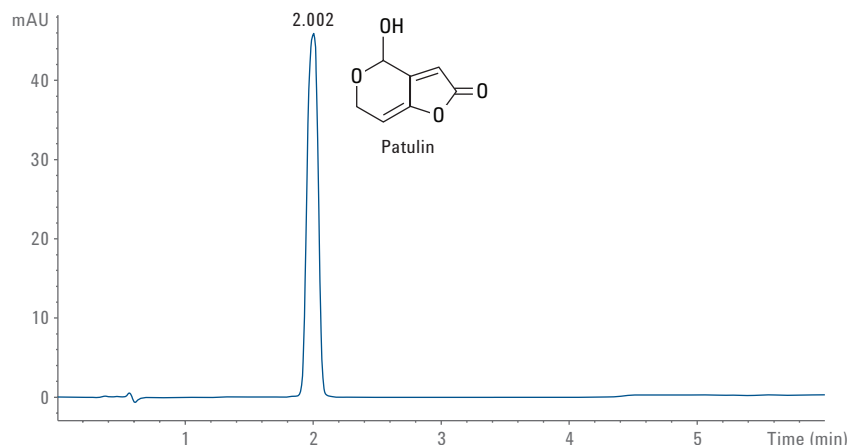


Figure 12. Determination of the mycotoxin patulin.

## Antioxidants

Antioxidants such as butylated hydroxyanisole (BHA, E320) and butylated hydroxytoluene (BHT) are primarily used as food additives. They act as scavengers and prevent free radical reactions. Propyl gallate (PG, E310) is used as an additive in oily and fatty food to reduce oxidation. For unsaturated vegetable oils, tert-butylhydroquinone (TBHQ, E319) is a very effective antioxidant that enhances storage lifetime.

Parameter	Value
Test sample	PG, BHT, BHA, TBHQ, 10 mg each, dissolved in 100 mL acetonitrile
Gradient	10 to 95% B in 10 minutes
Stop time	10 minutes
Post time	5 minutes
Flow rate	0.5 mL/min
Injection volume	1 µL
Wash	6 seconds with methanol
Column temperature	40 °C
DAD	240/4 nm, Ref. 360/100 nm, data rate 10 Hz, path length 10 mm, slit 8 nm

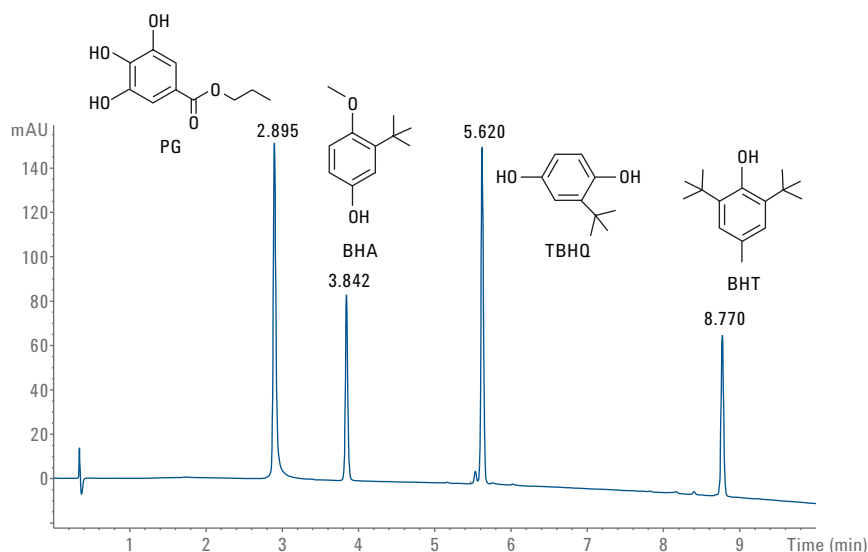


Figure 13. Separation of PG, BHA, BHT, and TBHQ, used as antioxidation additives in foods and cosmetics.

## Conclusions

This Application Note demonstrates the capability of the Agilent 1290 Infinity II Multi-Method Solution to support multiple LC methods on one system (multimethod analysis) as needed, for example, in food analysis laboratories. Equipped with the Agilent 1290 Infinity II Multicolumn Thermostat, this configuration provides access to up to eight different columns. In addition, with two solvent selection valves attached to the Agilent 1290 Infinity II Flexible Pump, the configuration runs up to 26 different solvents as mobile phase. Hence, it is possible to create more than 1,000 different separation methods. All LC methods can be run automatically in one sequence by an automated switch of columns and solvents. The large number of samples is handled by the Agilent 1290 Infinity II Multisampler.

## References

1. Anon. Performance Characteristics of the Agilent 1290 Infinity II Multicolumn Thermostat; Technical overview, Agilent Technologies, Inc. Publication number 5991-5533EN, **2015**.
2. Anon. Performance Characteristics of the Agilent 1290 Infinity II Multisampler; Technical overview, Agilent Technologies, Inc. Publication number 5991-5348EN, **2014**.



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