Agilent 1290 Infinity Multi-method Solution

Analysis of seven different food applications on one instrument – no column change, no mobile phase change

Application Note

Food

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Abstract

The Agilent 1290 Infinity Multi-method Solution for LC offers:

- Automated switching between up to eight columns of 100 mm or shorter in length, or up to six columns of 300 mm or shorter in length with an additional bypass and waste-line, respectively.
- Solvent selection – switching of up to 26 solvents using two solvent-selection valves.
- Over 1000 different separation conditions can be configured without further user interaction – depending upon column and solvent combination.
- The ability to specify the column and the mobile phases as method parameters. This allows different methods with different columns and mobile phases to be configured on one instrument without user interaction. This also allows these methods to be part of a sequence.
- A system that is ready for use whenever needed. Installation of column and solvents is complete.

Agilent Technologies

Introduction

Frequently, many applications are performed in the same laboratory, using different columns, mobile phases, column temperatures, and other chromatographic parameters. Typically, this involves a change of the column or change of mobile phases or both, which is time consuming. In addition, the risk of bad column connections increases with the number of installations. Change of mobile phase is often needed due to the different pH requirements of different organic phases.

The Agilent 1290 Infinity Multi-method Solution for LC offers:

- Automated switching between up to eight columns of 100 mm or less in length, or up to six columns of 300 mm or less in length with an additional bypass and waste-line, respectively.
- Solvent selection, which includes switching for up to 26 solvents.
- Over 1000 different separation conditions without further user interaction, depending on the column and solvent combination.

Using this system, the column and the mobile phases become method parameters. This allows different methods with different columns and mobile phases to be set up on one instrument, without user interaction. In addition, these methods can be part of a sequence.

In this Application Note we demonstrate how to set up such a system. We have chosen different applications from a food control lab as examples.

Experimental

Equipment

The Agilent 1290 Infinity Multi-method Solution consisted of:

- Two solvent selection valves
- Agilent 1290 Infinity Pump
- Agilent 1290 Infinity Autosampler
- Agilent 1290 Infinity Column Compartments
- Agilent 1290 Infinity Diode Array Detector and Agilent 6140 Single Quadrupole LC/MS system
- Several Agilent ZORBAX RRHT 1.8 µm columns
- One Agilent Poroshell 120 column
- Agilent ChemStation B04.02 software

Results and discussion

Configuring the system

1. Open the *Instrument Configuration* screen (Figure 1).
2. Click on the *Auto Configuration* button to initiate the search for available modules. The software typically asks for the IP address of the DAD. If this is provided the configuration of column compartments and solvent selection valves can begin.

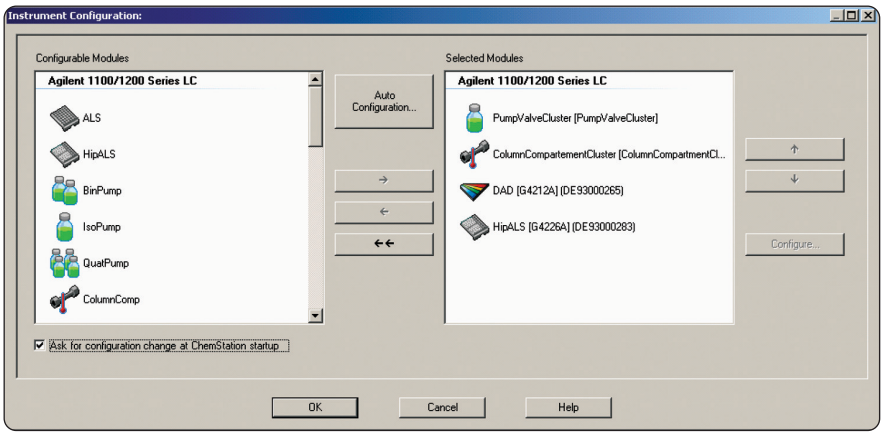


Figure 1
Auto configuration screen.

3. Highlight the *column compartment* and click on *Configure* to start the compartment clustering screen (Figure 2).

In this screen the high pressure column switching valve is clustered with the appropriate column compartment. The low pressure column switching valve is installed in column compartment 1 and the high pressure column switching valve is installed in column compartment 2. The high pressure valve inlet port is connected to the injection valve and the low pressure valve outlet port is connected to the detector.

The solvent selection valves must be clustered with the pump by highlighting the pump and clicking on *Configure* (Figure 1). In Figure 3 the clustering screen for the solvent selection valve is shown.

Valve 2 is clustered with Channel A1, the channel for the aqueous mobile phases. Valve 1 is clustered with Channel B1, which is used for the organic phases.

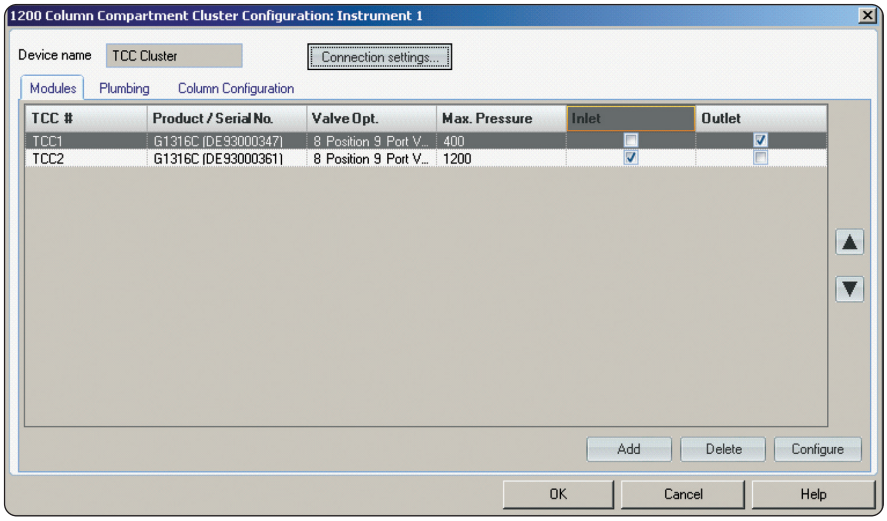


Figure 2
Compartment clustering screen.

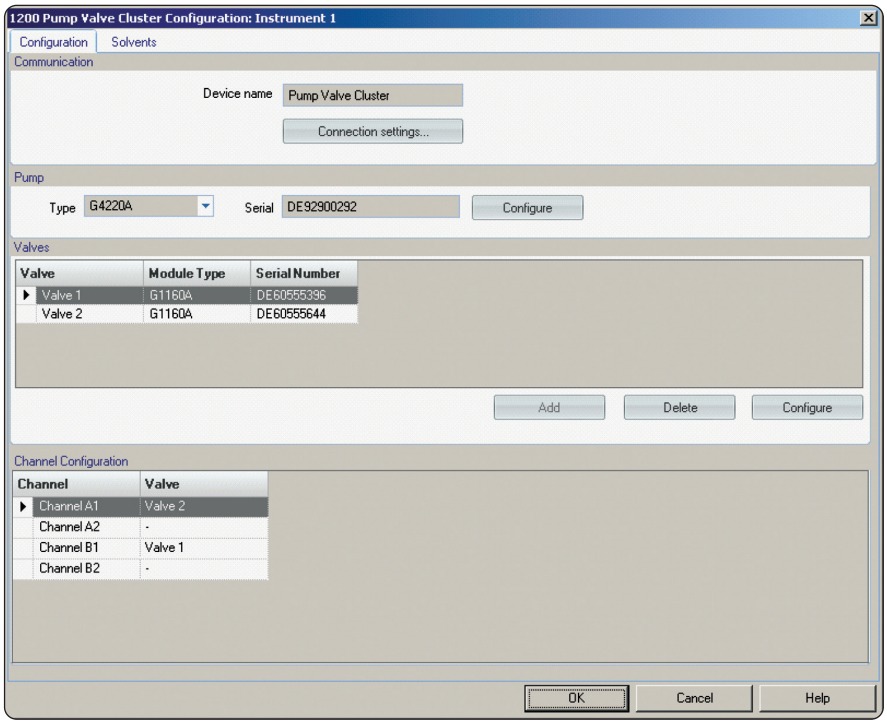


Figure 3
Solvent selection and pump clustering screen.

Application Example

The following application example was chosen to demonstrate the use of a multi application system. In this example, we have selected typical applications from food control laboratories such as the analysis of:

- Preservatives
- Antioxidants
- Coloring additives
- Mycotoxins
- Antimicrobial drugs
- Antiparasitic drugs

For each application, different columns, mobile phases, column temperatures, detector settings, flow rates, and gradients must be used. In Table 1, a matrix is shown with differences in column, mobile phases and detection. This matrix is used to set up the column compartments and the solvent selection valves.

Compounds and MW	Column and serial number	Mobile phase A	Mobile phase B	Temperature	MS ionization
Preservatives Benzoic acid 121 Salicylic acid 138 Citric acid 192	Agilent ZORBAX Eclipse C 8 100 mm × 2.1 mm USUUB01164	Water + 0.05% TFA pH=2.1	ACN:Isopropanol = 1:1 + 0.05% TFA	40 °C	UV detection only
Parabens Methyl 152 Ethyl 166 Propyl 180 Butyl 194	Agilent Poroshell 120 C-18 100 mm × 3.0 mm USCFX01019	Water + 5 mM NH_4OAc pH= 6.73	MeOH	45 °C	ESI negative
Malachite green 329.2	Agilent ZORBAX SB C18, 2.1 mm × 50 mm USWEY04101	Water +formic acid 0.1% pH=2.7	ACN + 0.1% FA	40 °C	ESI positive
Sudan red Sudan 1 249 Sudan 2 277 Sudan 3 353 Sudan orange G 215	Agilent ZORBAX Eclipse plus C18, 2.1 mm × 100 mm USDAZ01033	Water + formic acid 0.1% pH = 2.7	ACN + 0.1% FA	30 °C	ESI positive
Chloramphenicol 231	Agilent ZORBAX SB C8 2.1 mm × 100 mm USHDF01282	Water	Methanol	30 °C	ESI negative
Patulin 153	Agilent ZORBAX Extend 2.1 mm × 100 mm USHBE01536	0.1% NH_4OH pH=10.3	ACN + 0.1% NH_4OH	40 °C	ESI negative
Antioxidants PG 211 TBHQ 165 BHA 179 BHT 219 THBP 196.2	Agilent ZORBAX Eclipse C18 2.1 mm × 50 mm or SB Phenyl USSPK01256	Water + 0.05% acetic acid pH = 3.43	Acetonitrile	40 °C	ESI negative

Table 1
Application matrix.

Configuring columns and solvents

The column switching valve has eight positions, which can be connected to a column or used as bypass or waste positions. In our example, seven columns were installed and one position was used as bypass position. Each column is color-coded and the valve position is defined in the set up screen (Figure 4). In this screen, the valve positions are dedicated to hold a column or to be used as bypass or waste position. The column positions in the column compartments are color-coded and shown on the right side of the screen.

Colored clips are used to hold the columns. All capillaries are labeled with colored plastic rings for error-free identification of which column is attached to which valve position.

Next, each color-coded position is assigned a specific column (Figure 5). The columns must be entered into a database before a specific column can be selected from this screen. This database stores column parameters such as maximum temperature and pH range.

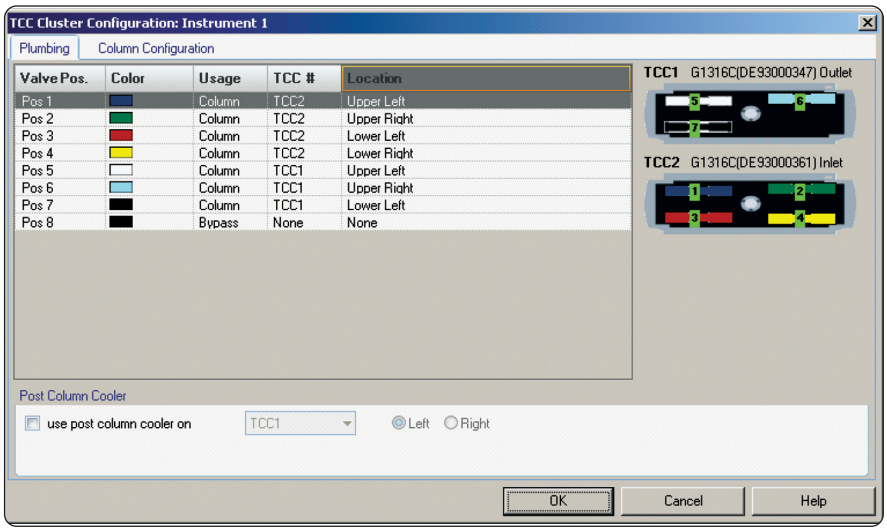


Figure 4
Assigning valve positions, colors, and column compartments to be equipped with a "column" or used as "bypass" positions.

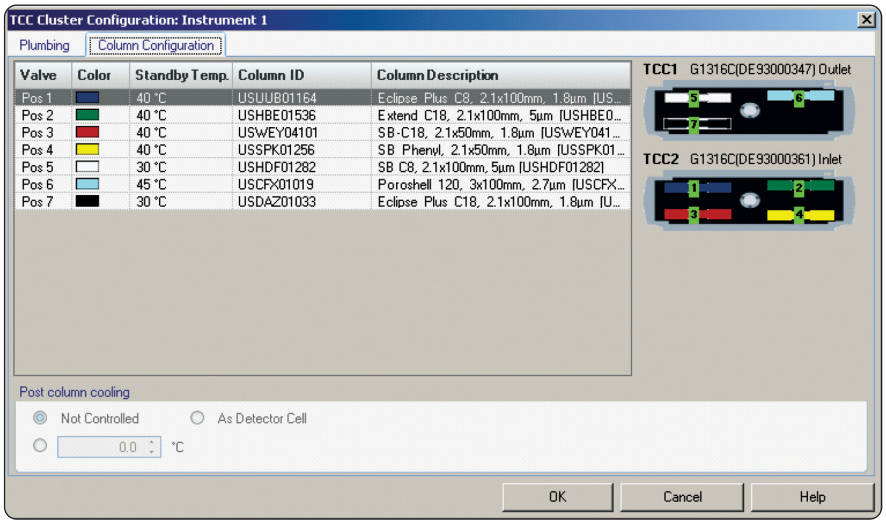


Figure 5
Assigning specific columns to the color-coded valve positions.

At the same time, a Standby Temp can be added for each column. The serial number identifies each individual column. This is especially important if two identical columns must be used. The column dimensions and the packing material are also stored.

The mobile phases must then be assigned to an appropriate valve position of the solvent selection valves. This is done using the screen shown in Figure 6.

Six aqueous mobile phases are available from valve 2. Seven organic mobile phases are available from valve 1. After this last configuration step, method setup can begin.

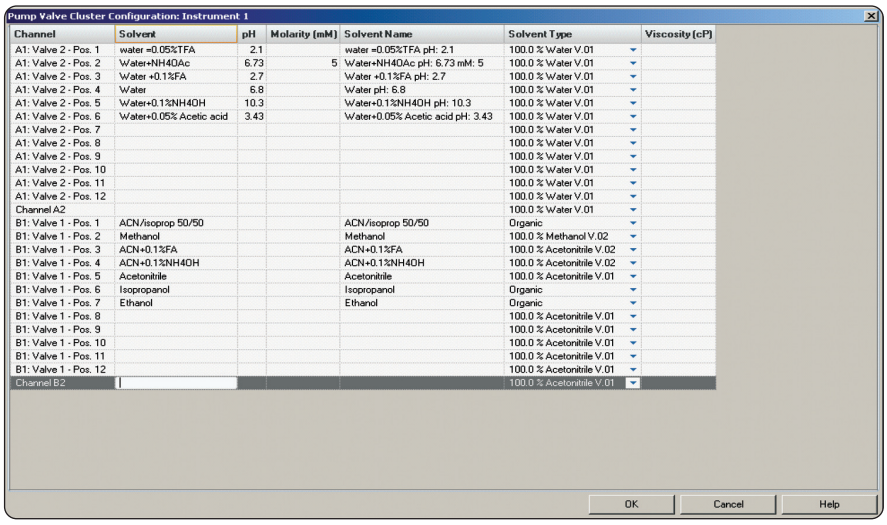


Figure 6
Assigning aqueous and organic solvents to the appropriate valve and to a valve position.

Setting up chromatographic methods

While setting up a chromatographic method, the selection of column and mobile phases is done from the instrument setup screens (Figures 7 and 8). No further user interaction with the column switching valve or solvent selection valve is needed.

In addition to the chromatographic parameter shown in Figure 7, the aqueous and organic mobile phases needed for a specific separation screen are selected.

In Figure 8, the column is chosen by clicking the buttons on the left side of the screen. The position of the selected column in the column compartments is shown on the right side of the screen.

Column and mobile phase are now method parameters. In Figure 9, the appropriate sections of a method printout are shown.

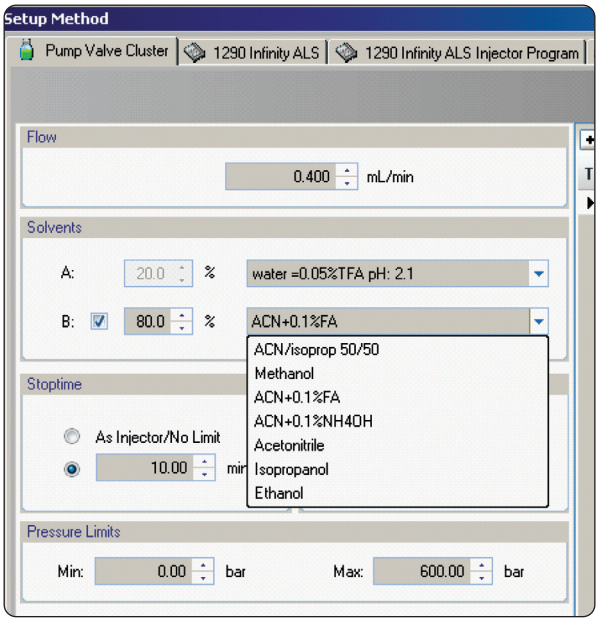


Figure 7
Selection of mobile phases for a chromatographic method.

Precautions

- To maintain the life of the column, it is recommended to flush the bypass from pump to detector with the new mobile phase before switching the needed column into the flow path. This protects the column from a solvent with a pH that is not recommended for this column.
- It is also recommended to clean the column after use, with a solvent that is best suited for longest life of the column, especially if the column is not used for several days.
- It is also recommended to use a column protection filter after the injection valve and before the column. If sample particles block the filter, the filter can be changed and the column can still be used.
- A convenient solution is to set up a *Master sequence for each application* that takes care of flushing, equilibration and column storage (Figure 10).
- In the first line, the system is flushed (column switching valves in Bypass) with solvents that are needed for the selected application.
- In the second line, the column is switched into the flow path and is equilibrated with the start conditions.
- In the third line a blank run is started.
- In the fourth line the standard is analyzed.
- In the fifth line the sample is analyzed.
- In the sixth line the column is cleaned for storage.
- Needed lines can be added, deleted, changed, or saved using an appropriate new name.

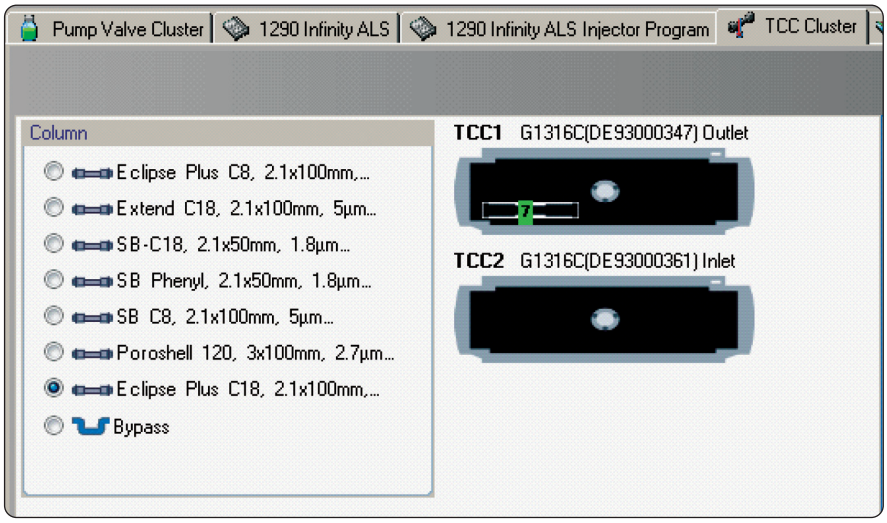


Figure 8
Selection of a column for a chromatographic method.

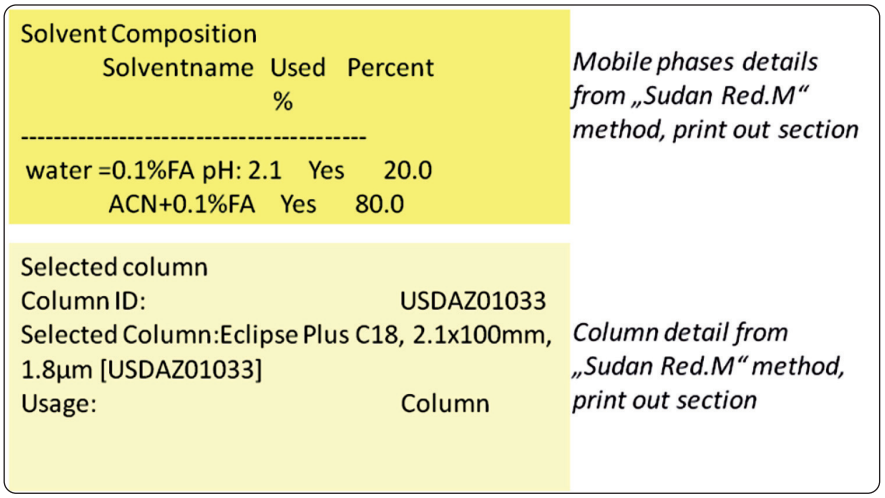
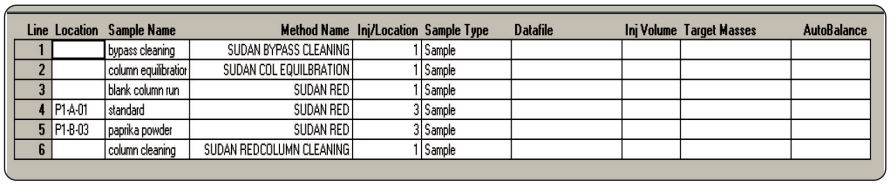


Figure 9
Method details for selected column and mobile phases from a method printout.



Line	Location	Sample Name	Method Name	Inj/Location	Sample Type	Datafile	Inj Volume	Target Masses	AutoBalance
1		bypass cleaning	SUDAN BYPASS CLEANING	1	Sample				
2		column equilibration	SUDAN COL EQUILBRATION	1	Sample				
3		blank column run	SUDAN RED	1	Sample				
4	P1-A-01	standard	SUDAN RED	3	Sample				
5	P1-B-03	paprika powder	SUDAN RED	3	Sample				
6		column cleaning	SUDAN REDCOLUMN CLEANING	1	Sample				

Figure 10
Master sequence for Sudan red application.

Once all the methods and master sequences are set up, the above mentioned applications can be performed without the need to change columns or mobile phases. The following applications were done one after the other, within half a day (Table 2). Sample preparation is not included in the half day time frame.

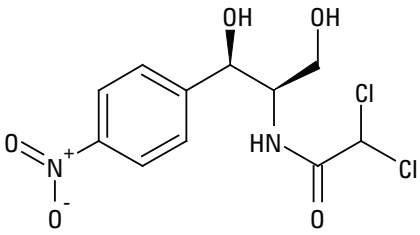
Application 1

Analysis of Chloramphenicol in Honey

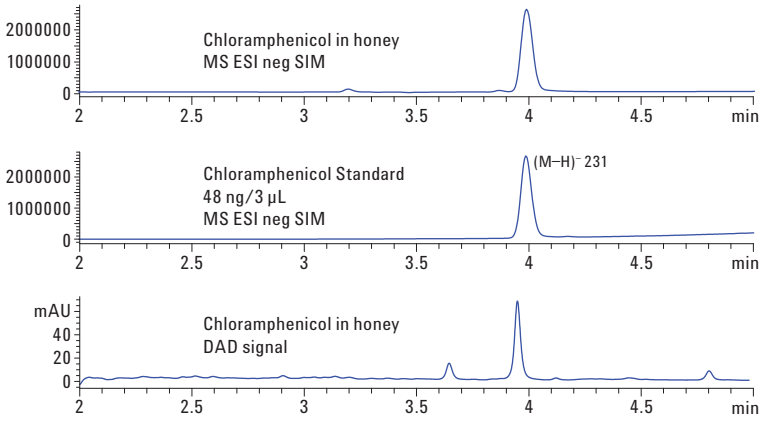
Compound/Compound Class

Chloramphenicol is an antibacterial, antimicrobial drug. It is a broad-spectrum antibiotic and is used to protect animals and insects such as bees. Therefore, this compound can be found in honey.

A side effect is bone marrow toxicity.



Chromatogram and Conditions



Test Sample

Chloramphenicol, Water/Methanol = 9:1

Sample preparation: 1 g of honey was diluted with 4 ml of water, The liquid phase was filtered through a 0.45-µm Econofilter from Agilent and spiked with Chloramphenicol to a concentration of 48 ng/3 µL

Column Agilent ZORBAX RRHT SB C8, 100 mm × 2.1 mm, 1.8 µm for 600 bar operation

Pump

Solvent A: H_2O
Solvent B: Methanol
Gradient: 10 to 90% B in 8 min, Stop Time 10 min , Post time 4 min
Flow Rate: 0.3 mL/min

Autosampler with 40 µl injection loop

Injection volume: 3 µL
Wash: 10 s for exterior of needle

Thermostatted Column Compartment

Temperature: 30 °C

DAD

10 mm path length, Wavelength 220/4 nm and 254/10 nm Ref 360/100 nm, 20Hz

MSD Multi mode

ESI negative, SIM Ion = 231, Drying gas 5 L/min, Vaporizer Temp 150 °C, Nebulizer pressure 60 psig, V_{cap} positive and negative and V_{charge} positive and negative 2000 V, Corona 5 µA

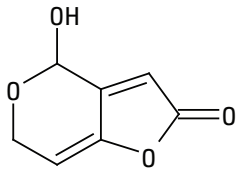
Application 2

Analysis of Patulin in Apple Juice

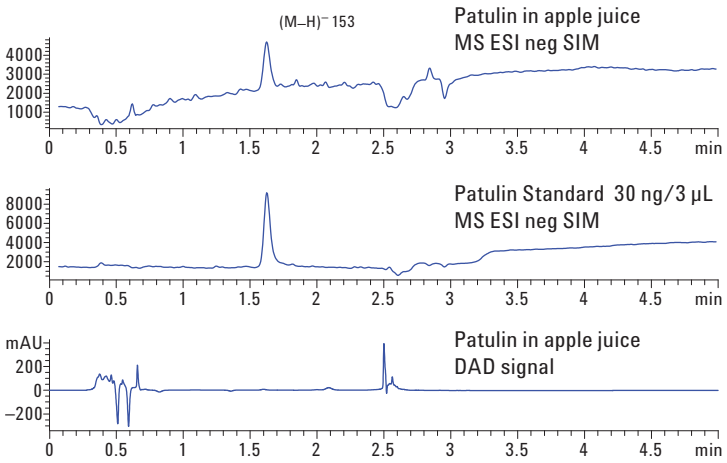
Compound/Compound Class

Patulin is a mycotoxin and found in rotting apples. It is suspected to be genotoxic. Several countries have restrictions on apple products. The World Health Organization recommends a maximum concentration of 50 µg/L in apple juice.

In the European Union the limit is set to 50 µg/kg in apple juice and cider, 25 µg/kg in solid apple products, and 10 µg/kg in products for infants and young children. These limits were set on November 1, 2003.



Chromatogram and Conditions



Test Sample
Patulin, in apple juice
Sample preparation: 1 mL of apple juice was spiked with 10 µg Patulin

Column Agilent ZORBAX RRHT Extend, 100 mm × 2.1 mm, 1.8 µm for 600 bar operation

Pump
Solvent A: H_2O + 0.1% NH_4OH
Solvent B: ACN + 0.1% NH_4OH
Gradient: 1% for 1.8 min to 90% B in 2.3 min, Stop Time 5 min, Post time 3 min
Flow Rate: 0.5 mL/min

Autosampler with 40 µL injection loop
Injection volume: 3 µL
Wash: 10 s for exterior of needle

Thermostatted Column Compartment
Temperature: 40 °C

DAD
10 mm path length, Wavelength 220/4 nm and 254/10 nm Ref 360/100 nm, 20 Hz

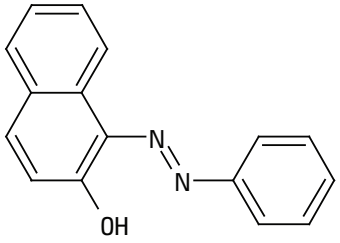
MSD with Multimode Source
ESI negative, $(M–H)^-$ 153, Drying gas 5 L/min, Vaporizer Temp 150 °C, Nebulizer pressure 60 psig, V_{cap} positive and negative and V_{charge} positive and negative 2000 V, Corona 5 µA

Application 3

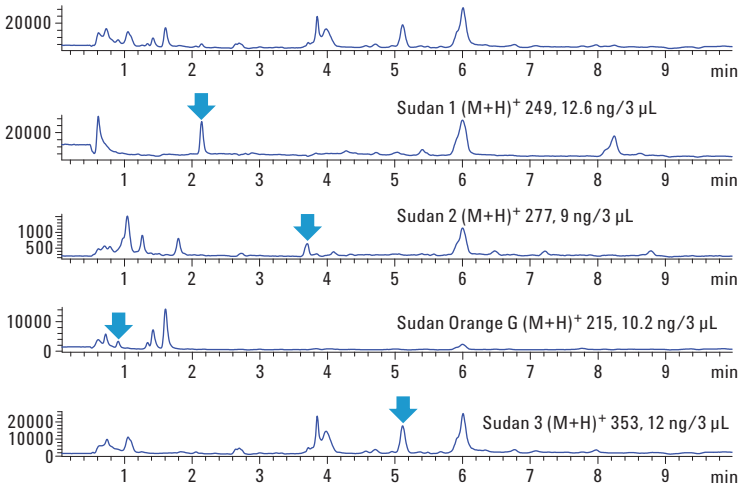
Analysis of Sudan Red in Paprika Powder

Compound/Compound Class

Sudan I is a powdered substance with an orange-red appearance. Sudan I has also been used for coloring food products, such as curry powder and chili powder. However, the use of Sudan I in foods is now banned in many countries because Sudan I, Sudan III, and Sudan IV have been classified carcinogens by the International Agency for Research on Cancer.



Chromatogram and Conditions



Test Sample
Sudan red 1, 2, 3, orange G

Sample preparation:	Extract was spiked to a concentration of Sudan 1 = 12.6 ng/3 µL, Sudan 2= 9 ng/3 µL, Sudan 3 = 12 ng/3 µL, Sudan orange G = 10.2 ng/3 µL , 2.5 g amount of paprika powder was extract ed with 20 mL of acetonitrile and left for 45 minutes in the ultra sonic bath. The liquid phase was filtered through a 0.45-µm Econofilter from Agilent.
Column	Agilent ZORBAX RRHT Eclipse Plus C18, 100 mm × 2.1 mm, 1.8 µm for 600 bar operation

Pump

Solvent A:	H_2O + 0.1% FA
Solvent B:	ACN + 0.1% FA
Gradient:	80% to 95% B in 10 min, Stop Time 4 min, Post time 1 min
Flow Rate:	0.4 mL/min

Autosampler with 40 µl injection loop

Injection volume:	5 µL
Wash:	10 s for exterior of needle

Thermostatted Column Compartment

Temperature:	30 °C

DAD
10 mm path length, Wavelength 220 mm, 10 Hz

MSD Multimode
ESI negative, SIM ions $(M+H)^+$ 249, 277, 353, 215, Drying gas 5 L/min, Vaporizer Temp 150 °C, Nebulizer pressure 60 psig, V_{cap} positive and negative and V_{charge} positive and negative 2000 V, Corona 5 µA

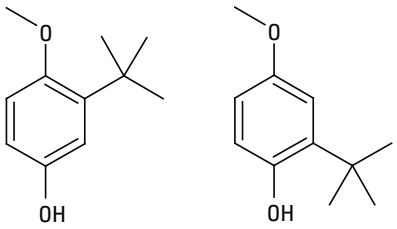
Application 4

Analysis of Antioxidants

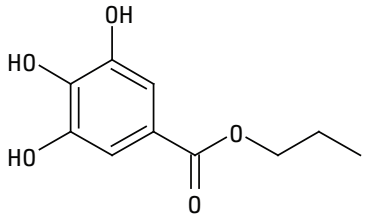
Compound/Compound Class

Antioxidants Butylated hydroxyanisole (BHA) is an antioxidant used as a food additive with the E number E320. Usually the intake levels of BHA and BHT is very low.

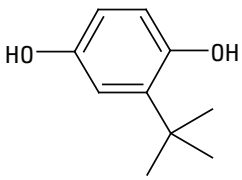
Butylated hydroxytoluene (BHT), BHA, and Trihydroxy-butyrophenon (THBP) are able to stabilize free radicals. By acting as free radical scavengers, other free radical reactions are prevented.



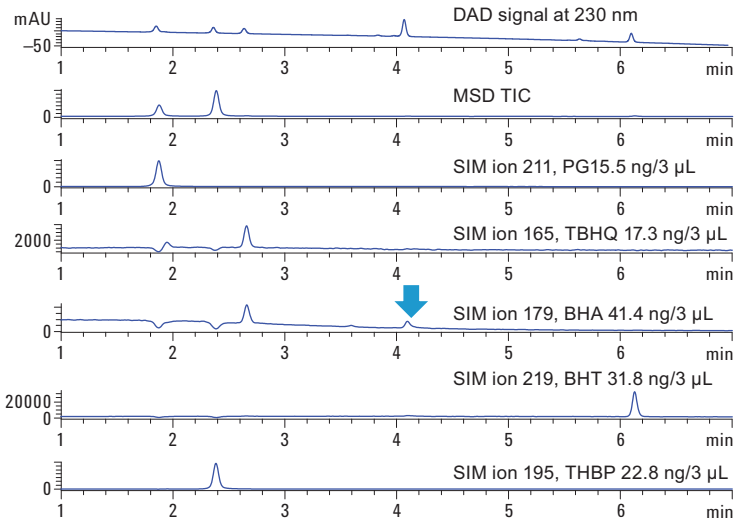
Propyl gallate, or propyl 3,4,5-trihydroxybenzoate has been used since 1948 as an antioxidant, and is added to foods containing oils and fats to reduce oxidation. As a food additive, it is used under the E number E310.



TBHQ (tert-butylhydroquinone) is a very effective preservative for unsaturated vegetable oils. It is sometimes combined with other preservatives such as butylated hydroxyanisole (BHA). As a food additive, its E number is E319. It is added to a wide range of foods, with the highest limit (1000 mg/kg). TBHQ as a food additive is regulated in the. United States by 21 C.F.R. § 172.185. Its primary advantage is enhancing storage life.



Chromatogram and Conditions



Test Sample
BHA; BHT; THBP; PG; TBQHQ; dissolved in methanol

Column	Agilent ZORBAX RRHT SB phenyl, 50 mm × 2.1 mm, 1.8 µm for 600 bar operation

Pump

Solvent A:	H_2O + 0.5% Acetic acid
Solvent B:	ACN
Gradient:	10% to 95% B in 10 min, Stop Time 7 min, Post time 3 min
Flow Rate:	0.5 mL/min

Autosampler with 40 µL injection loop

Injection volume:	3 µL
Wash:	10 s for exterior of needle

Thermostatted Column Compartment

Temperature:	40 °C

DAD
10 mm path length, Wavelength 230/4 mm, Ref 360/100 nm, 20 Hz

MSD Multi mode
ESI negative, SIM ions 211, 165, 195, 179, 219, Drying gas 5 L/min, Vaporizer Temp 150 °C, Nebulizer pressure 60 psig, V_{cap} positive and negative and V_{charge} positive and negative 2000 V, Corona 5 µA

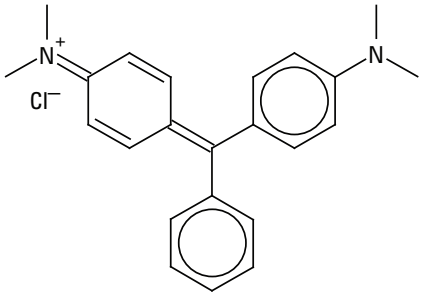
Application 5

Analysis of Malachite Green

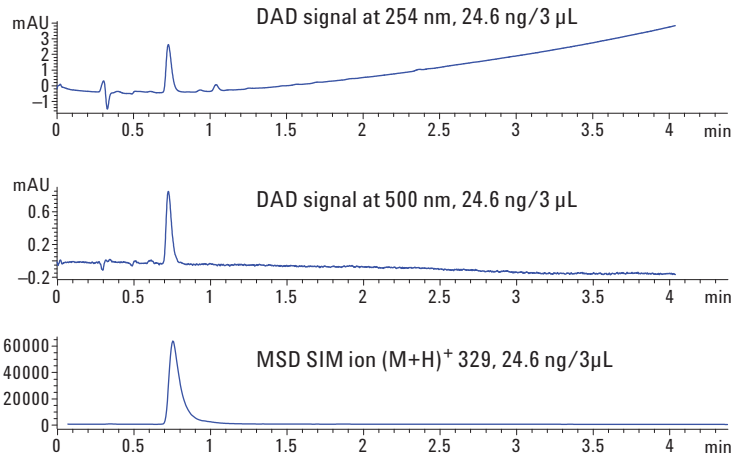
Compound/Compound Class

Malachite green is used as a topical antiseptic or to treat parasites, fungal infections, and bacterial infections in fish and fish eggs. It is also used as a bacteriological stain.

In 1992 it was determined that there is a significant health risk to humans who eat fish contaminated with malachite green. The substance has been banned in the United States since 1983 in food-related applications.



Chromatogram and Conditions



Test Sample
Malachite green in Acetonitrile. Diluted such that a concentration of 24.6 ng/3 µL was obtained

Column	Agilent ZORBAX RRHT SB C18, 50 mm × 2.1 mm, 1.8 µm for 600 bar operation

Pump

Solvent A:	H_2O + 0.1% FA
Solvent B:	ACN + 0.1% FA
Gradient:	60% to 95% B in 3 min, Stop Time 5 min, Post time 3 min
Flow Rate:	0.4 mL/min

Autosampler with 40 µL injection loop

Injection volume:	3 µL
Wash:	10 s for exterior of needle

Thermostatted Column Compartment

Temperature:	40 °C

DAD
10 mm path length, Wavelength 254/10 nm and 500/20 nm, Ref = off, 20 Hz

MSD Multi mode
ESI negative, SIM ions 329.2, Drying gas 5 L/min, Vaporizer Temp 150 °C, Nebulizer pressure 60 psig, V_{cap} positive and negative and V_{charge} positive and negative 2000 V, Corona 5 µA

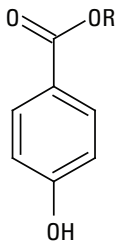
Application 6

Analysis of Parabens

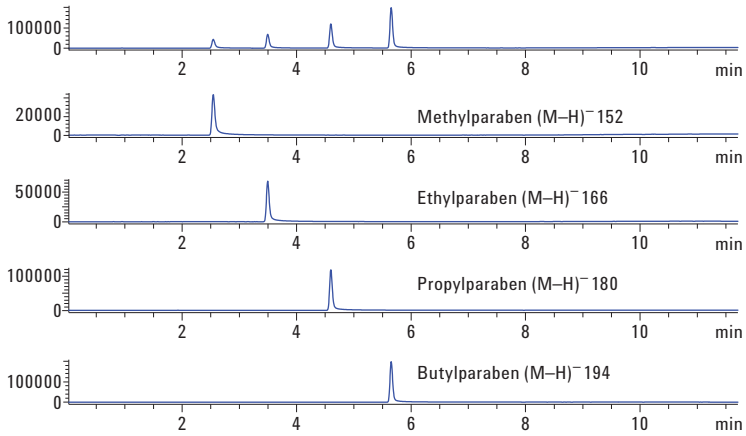
Compound/Compound Class

Parabens are widely used as preservatives in the cosmetic and pharmaceutical industries. They are used in shampoos, commercial moisturizers, shaving gels, personal lubricants, spray tanning solution and toothpaste. They are also used as food additives

Common parabens include methylparaben (E number E218), ethylparaben (E214), propylparaben (E216) and butylparaben.



Chromatogram and Conditions



Test Sample
Methyl, ethyl, propyl, butylparaben, 100 ng/mL in Methanol/water = 1/1

Column	Agilent ZORBAX Poroshell 120 C18, 100 mm × 3.0 mm, 2.7 µm for 600 bar operation

Pump

Solvent A:	H_2O + 5% mM NH_4OAc
Solvent B:	Methanol
Gradient:	40% to 55% B in 5 min, Stop Time 12 min, Post time 3 min
Flow Rate:	0.5 mL/min

Autosampler with 40 µL injection loop

Injection volume:	3 µL
Wash:	10 s for exterior of needle

Thermostatted Column Compartment

Temperature:	45 °C

DAD
10 mm path length, Wavelength 254 nm, 10 Hz

MSD Multi mode
ESI negative, SIM ions 152, 166, 180, 194, Drying gas 5 L/min, Vaporizer Temp 150 °C, Nebulizer pressure 60 psig, V_{cap} positive and negative and V_{charge} positive and negative 2000 V, Corona 5 µA

Application 7

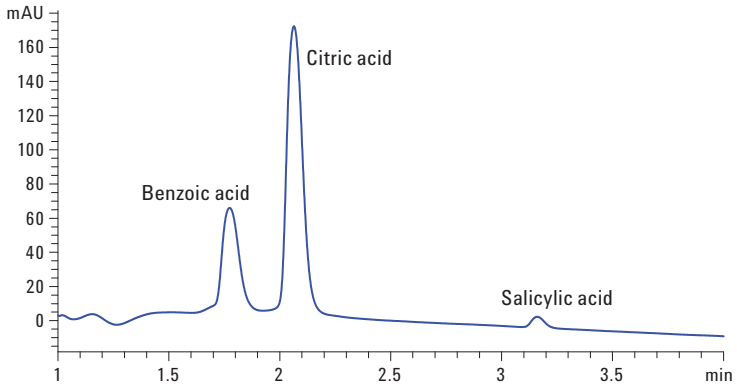
Analysis of Preservatives

Compound/Compound Class

Preservatives

Sodium benzoate E211, citric acid E330, and E200 sorbic acid are used as preservatives in food and drinks to prevent the growth of mold, yeast and fungi.

Chromatogram and Conditions



Test Sample

Sorbic acid, benzoic acid 6.5 mg/5 mL, salicylic acid 4.3 mg/5 mL, citric acid 11.8 mg/5 mL, 5 mg/10 mL in methanol, 1:100 dilute with methanol

Column	Agilent ZORBAX RRHT Eclipse C8, 100 mm × 2.1 mm, 1.8 µm for 600 bar operation

Pump

Solvent A:	H_2O + 0.05% TFA
Solvent B:	ACN/Isopropanol 50/50 + 0.05% TFA
Gradient:	35% to 75% B in 10 min, Stop Time 10 min, Post time 5 min
Flow Rate:	0.3 mL/min

Autosampler with 40 µL injection loop

Injection volume:	3 µL
Wash:	10 s for exterior of needle

Thermostatted Column Compartment

Temperature:	45 °C

DAD

10 mm path length, Wavelength 205/4 mm, Ref 360/100 nm, 10 Hz

Conclusions

The Agilent 1290 Infinity Multi-method Solution for LC provides:

- The ability to specify column and mobile phases as method parameters
- Multi-applications on one instrument using up to seven columns in two column compartments, plus one bypass cleaning path
- The ability to set up master sequences for each application with flushing, equilibration, and column care steps
- Elimination of time consuming column change and mobile phase change steps
- An Agilent 1290 Infinity LC system prepared for all setup applications whenever needed
- Easy addition of new applications by adding more solvents and using one of the installed columns.

www.agilent.com/chem/1200mds

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