Different Food Applications on a Single LC System Using Automated Column and Solvent Selection

Agilent 1260 Infinity II Multimethod Solution

Application Note

Food Testing & Agriculture

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Abstract

The Agilent 1260 Infinity II Multimethod solution facilitates running multiple LC applications on one LC system without manual system changes. Automated solvent selection typically using up to 15 different mobile phases, and automated column switching between up to four columns enables the use of more than 100 different separation conditions. Columns and solvents are specified as method parameters, allowing different methods with different columns and solvents to be part of a sequence. This Application Note describes the use of the 1260 Infinity II Multimethod solution for the analysis of antioxidants, sweeteners, and preservatives as application examples from a food control laboratory.

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Introduction

In many laboratories, different applications requiring different columns, mobile phases, and other chromatographic parameters are performed on the same LC system. This usually necessitates the manual exchange of columns and mobile phases to adapt the instrument to the desired application. The Agilent 1260 Infinity II Multimethod solution facilitates automated solvent selection typically using up to 15 different mobile phases and automated column switching between up to four columns of 300 mm or less in length. This setup enables the use of more than 100 different separation conditions without manual system changes. If switching between a larger number of columns and mobile phases is required, the Agilent 1290 Infinity II Multimethod solution, described in a previous Application Note[1], is the instrument of choice.

Food additives are substances that are added to food or involved in production, processing, packaging, or storage to increase the nutritional or sensory value, or prolong shelf life[2]. Examples of food additives include vitamins, aroma substances, sweeteners, colors, preservatives, and antioxidants, among others. In most countries, the use of food additives is regulated. In the European Union, substances that are used as food additives are coded with an E number.

Antioxidants, such as BHA (E 320) and BHT (E 321) are used to retard lipid oxidation and thereby avoid food deterioration caused by the generation of undesirable aromas due to lipid degradation products[2]. Sweeteners, such as acesulfam K (E 950) and saccharin (E 954), are compounds that generate a sweet sensation, but possess negligible nutritional value[2]. Preservatives or antimicrobial agents are needed to prolong shelf life if the elimination of microflora by physical methods is not possible. Weak acids, such as sorbic acid (E 200) and benzoic acid (E 210), are preferably used in acidic foods[2]. Salicylic acid is used as an antimicrobial agent in cosmetics and, apart from this, is also used as a keratolytic for the treatment of acne[3].

This Application Note describes the use of the 1260 Infinity II Multimethod solution for the analysis of antioxidants, sweeteners, and preservatives as application examples from a food control laboratory.

Experimental

Equipment

The Agilent 1260 Infinity II Multimethod solution comprised the following modules:

- Agilent 1260 Infinity II Quaternary Pump (G7111B)
- Agilent 1290 Infinity valve drive (G1170A) equipped with a solvent selection valve (G4235A)
- Agilent 1260 Infinity II Multisampler (G7167A) with a sample cooler (Option 100)
- Agilent 1260 Infinity II Multicolumn Thermostat (G7116A) with a column identification kit and valve drive (Option 058) equipped with a 4-column selection valve (G4237A) and capillary kit (Option 006)
- Agilent 1260 Infinity II Diode Array Detector HS (G7117C) with a 10-mm Max-Light cartridge cell, (G4212-60008)

Software

Agilent OpenLAB CDS ChemStation Edition, revision C.01.07 SR 2 [255]

Columns

- Agilent InfinityLab Poroshell 120 SB-C18, 4.6 × 100 mm, 2.7 µm (p/n 685975-902T)
- Agilent InfinityLab Poroshell 120 EC-C18, 4.6 × 100 mm, 2.7 µm (p/n 695975-902T)
- Agilent InfinityLab Poroshell 120 EC-C8, 4.6 × 100 mm, 2.7 µm (p/n 695975-906T)

Chemicals

All solvents were LC grade. Acetonitrile was purchased from Merck (Darmstadt, Germany). Fresh ultrapure water was obtained from a Milli-Q Integral system equipped with a 0.22-µm membrane point-of-use cartridge (Millipak, EMD Millipore, USA). Potassium dihydrogen phosphate was purchased from Merck (Darmstadt, Germany). Acetic acid, trifluoroacetic acid, propyl gallate, *tert*-butylhydroquinone, butylated hydroxyanisole, butylated hydroxytoluene, acesulfame K, sodium saccharin, and benzoic acid were obtained from Sigma-Aldrich (Steinheim, Germany). Aspartame and sorbic acid were purchased from Supelco (Bellefonte, USA). Salicylic acid and 2′,4′,5′-trihydroxybutyrophenone were obtained from Fluka (Buchs, Switzerland) and Synquest Laboratories (Alachua, USA), respectively.

Samples and methods

Analysis of antioxidants

Standards: A mixture of butylated hydroxyanisole (BHA, E 320), butylated hydroxytoluene (BHT, E 321), propyl gallate (PG, E 310), *tert*-butylhydroquinone (TBHQ, E 319), and 2′,4′,5′-trihydroxybutyrophenone (THBP) was prepared in water:acetonitrile (80:20, v:v) at a concentration of 10 µg/mL for PG and THBP, 50 µg/mL for BHA and TBHQ, and 100 µg/mL for BHT.

Sample and sample preparation: Chewing gum was bought in a local supermarket (Germany). One piece of chewing gum was cut into small pieces and extracted with 20 mL acetonitrile in an ultrasonic bath. Before analysis, the extract was filtered using a 1-mL plastic syringe with Captiva Premium syringe filters nylon, 15-mm, 0.45 µm (p/n 5190-5091).

Analysis of sweeteners

Standards: A mixture of acesulfam K (E 950), aspartame (E 951), and sodium saccharin (E 954) was prepared in water:acetonitrile (90:10, v:v) at a concentration of 10 µg/mL of each component.

Sample and sample preparation: A sugar-free energy drink was bought in a local supermarket (Germany). The sample was degassed by sonication, and filtered using a 1-mL plastic syringe with Captiva Premium syringe filters regenerated cellulose, 15-mm, 0.45 µm (p/n 5190-5109). For analysis, a 1:10 dilution of the sample with water was used.

Analysis of preservatives

Standards: A mixture of benzoic acid (E 210), salicylic acid, and sorbic acid (E 200) was prepared in water:acetonitrile (90:10, v:v) at a concentration of 10 µg/mL of each component.

Sample and sample preparation: Facial tonic was bought in a local supermarket (Germany). The sample was filtered using a 1-mL plastic syringe with Captiva Premium syringe filters regenerated cellulose, 15-mm, 0.45 µm (p/n 5190-5109). For analysis, a 1:1,000 dilution of the sample with water was used.

Table 1. Chromatographic conditions for analysis of antioxidants.

Parameter	Value
Column	Agilent InfinityLab Poroshell 120 SB-C18, 4.6 × 100 mm, 2.7 µm
Solvent	A) Water + 0.5 % acetic acid B) Acetonitrile
Gradient	10 %B at 0 minutes 95 %B at 10 minutes
Stop time	15 minutes
Post time	10 minutes
Flow rate	1.000 mL/min
Temperature	40 °C
Detection	280 nm/4 nm, Ref. 390 nm/20 nm, 20 Hz, spectra acquisition
Injection volume	10.0 µL
Sample temperature	10 °C

Table 2. Chromatographic conditions for analysis of sweeteners.

Parameter	Value
Column	Agilent InfinityLab Poroshell 120 EC-C18, 4.6 × 100 mm, 2.7 µm
Solvent	A) 20 mM KH_2PO_4 in water, pH 3 B) Acetonitrile
Gradient	15 %B at 0 minutes, 80 %B at 10 minutes
Stop time	10 minutes
Post time	5 minutes
Flow rate	1.000 mL/min
Temperature	30 °C
Detection	214 nm/4 nm, Ref. 360 nm/100 nm, 20 Hz, spectra acquisition
Injection volume	10.0 µL
Sample temperature	10 °C

Table 3. Chromatographic conditions for analysis of preservatives.

Parameter	Value
Column	Agilent InfinityLab Poroshell 120 EC-C8, 4.6 × 100 mm, 2.7 µm
Solvent	A) Water + 0.05 % trifluoroacetic acid B) Acetonitrile:isopropanol (50:50) + 0.05 % trifluoroacetic acid
Gradient	15 %B at 0 minutes, 40 %B at 10 minutes
Stop time	10 minutes
Post time	5 minutes
Flow rate	1.000 mL/min
Temperature	40 °C
Detection	235 nm/4 nm, Ref. 380 nm/40 nm, 20 Hz, spectra acquisition
Injection volume	10.0 µL
Sample temperature	10 °C

Instrument setup

The 1260 Infinity II Multimethod solution offers the ability to specify columns and solvents as method parameters. This enables different methods with different columns and solvents to run on one instrument without user interaction, and also allows these methods to be part of a sequence.

During instrument configuration, the solvent selection valve is clustered with the Agilent 1260 Infinity II Quaternary Pump to form a pump-valve cluster. Clustering of a solvent selection valve with a pump is shown in a previous Application Note[4]. In this Application Note, the solvent selection valve was connected to channel A of the 1260 Infinity II Quaternary Pump to enable solvent selection for the aqueous solvents used. In the Pump Valve Cluster Configuration menu, the positions of the solvent selection valve as well as channels B, C, and D of the 1260 Infinity II Quaternary Pump are named by the connected solvents. In the method of the pump-valve cluster, the solvents can then be chosen by name from a drop-down menu, and the solvent selection valve automatically switches to the corresponding position.

With the Agilent 1260 Infinity II Multicolumn Thermostat, a column identification tag reader (part of column identification kit) can be used to track column history. When installing columns with column identification tags, column properties such as a description, geometric data, as well as pH, pressure, and temperature limitations, are automatically available in the ChemStation columns table (Figure 1). Column history including the number of injections, maximum measured temperature, as well as first and recent injection date are tracked on the column identification tags.

In the Column Assignment dialog box (Figure 2), the plumbing of the 4-column selection valve is defined, and the columns installed are assigned to their respective positions. The Column Assignment dialog box is directly connected to the ChemStation columns table. Installed columns with column identification tag are automatically assigned to the correct location.

The 4-column selection valve offers four positions that can be connected to a column or used as a bypass or waste position. For this Application Note, three columns were installed, and one position was used as a bypass position.

For maximum column lifetime, it is recommended to flush the system from pump to detector through the bypass with the new mobile phase before the column needed is switched into the flow path. This protects the column from solvents with a pH value that is not recommended for this column. Additionally, it is recommended to flush the column with a solvent best suited for maximum column lifetime after use. An easy way to do this is to set up a master sequence for each application, which takes care of flushing, column equilibration, and column storage.

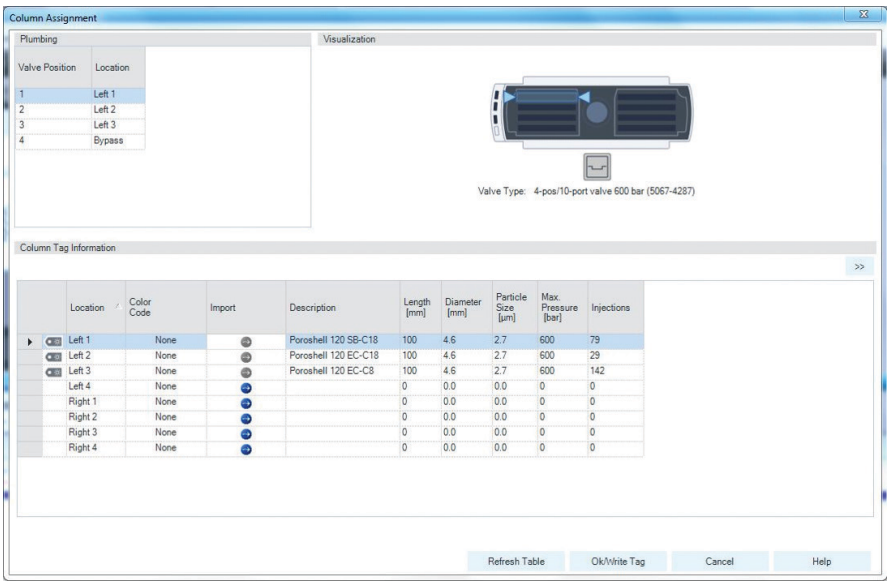


Figure 2. Column Assignment dialog box.

#	Installed	Location	Tag	Description	Col. Serial#	Batch#	Product#	# Injections	Max. P [bar]	Max. T [°C]	Max. pH	Min. pH	Length	Diameter	Size	Void	Unit	Comment
1	YES	Left 1	Sealed	Poroshell 120 SB-C18	USCEW03045	B16095	685975-902T	79	600	90.0	8.0	1.0	100.0	4.6	2.7	1.000	ml	
2	YES	Left 2	Sealed	Poroshell 120 EC-C18	USCFS07202	B16107	695975-902T	29	600	60.0	8.0	2.0	100.0	4.6	2.7	1.000	ml	
3	YES	Left 3	Sealed	Poroshell 120 EC-C8	USDFK01718	B16046	695975-906T	142	600	60.0	8.0	2.0	100.0	4.6	2.7	1.000	ml	

Figure 1. ChemStation columns table.

After column assignment, the column used in a specific method can be chosen from a drop-down menu in the Method dialog box of the 1260 Infinity II Multicolumn Thermostat (Figure 3). When a method is loaded, the 4-column selection valve is automatically switched to the position corresponding to the chosen column.

Results and Discussion

In this Application Note, analyses of antioxidants, sweeteners, and preservatives were performed as application examples from a food control laboratory using the 1260 Infinity II Multimethod solution. These analyses can be performed in a single sequence by selection of the column and solvents used as method parameters.

Analysis of antioxidants

Figure 4 shows the analysis of the antioxidants PG, THBP, TBHQ, BHA, and BHT and the retention time and area precision determined from 10 consecutive runs. Excellent retention time precision was obtained for all peaks. For PG, THBP, TBHQ, and BHT, area precision was compromised by degradation of these compounds in the standard mixture.

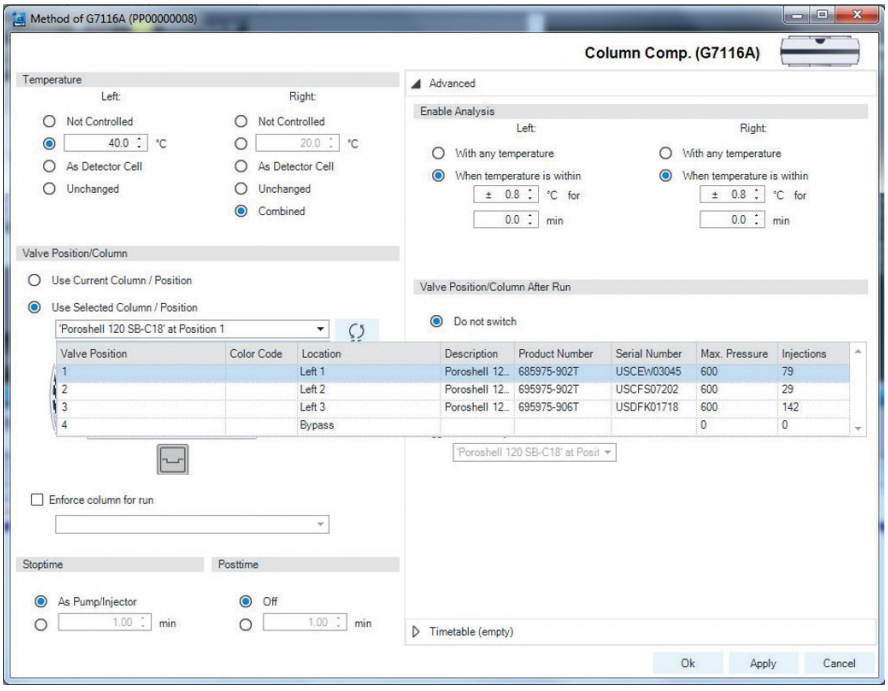
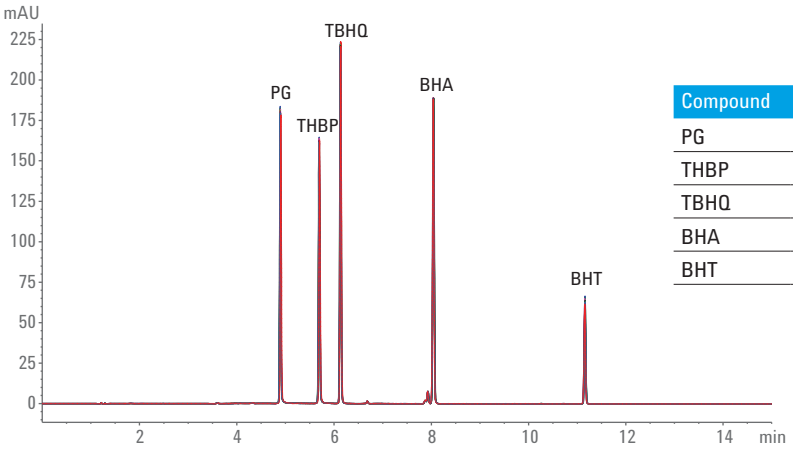


Figure 3. Column selection in the Method dialog box of the Agilent 1260 Infinity II Multicolumn Thermostat.



Compound	RT (min)	RT RSD (%)	Area	Area RSD (%)	Resolution
PG	4.90	0.09	298.6	0.92	–
THBP	5.70	0.07	302.7	0.67	17.7
TBHQ	6.13	0.05	422.7	0.56	8.9
BHA	8.04	0.03	398.9	0.11	36.1
BHT	11.16	0.03	136.2	2.18	55.9

Figure 4. Analysis of antioxidants: Overlay of 10 consecutive analyses of the standard mixture and determination of retention time and area precision.

Chewing gum was analyzed as an example for the analysis of a real sample. In the chewing gum sample, BHA could be identified based on the retention time and UV spectrum (Figure 5).

Analysis of sweeteners

Figure 6 shows the analysis of the sweeteners acesulfam, aspartame, and saccharin as well as the retention time and area precision determined from 10 consecutive runs. Excellent retention time and area precision were obtained for all peaks.

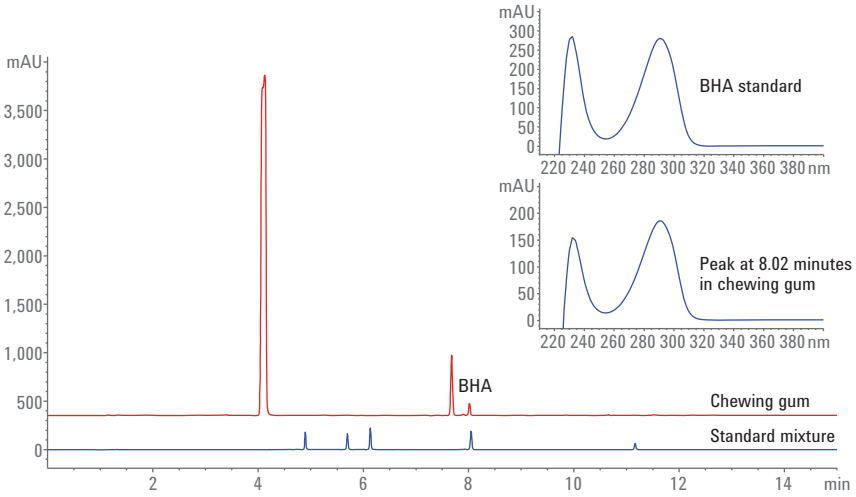
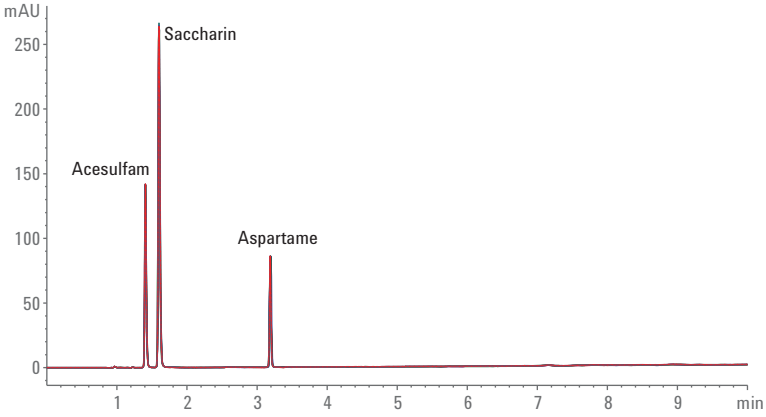


Figure 5. Analysis of antioxidants: Identification of BHA in chewing gum.



Compound	RT (min)	RT RSD (%)	Area	Area RSD (%)	Resolution
Acesulfam	1.41	0.10	222.4	0.08	–
Saccharin	1.60	0.14	471.0	0.09	4.5
Aspartame	3.19	0.12	130.1	0.08	37.2

Figure 6. Analysis of sweeteners: Overlay of 10 consecutive analyses of the standard mixture and determination of retention time and area precision.

A sugar-free energy drink was analyzed as an example for the analysis of a real sample. In the energy drink sample, acesulfam and aspartame could be identified based on the retention time and UV spectrum (Figure 7).

Analysis of preservatives

Figure 8 shows the analysis of the preservatives sorbic acid, benzoic acid, and salicylic acid as well as the retention time and area precision determined from 10 consecutive runs. Excellent retention time and area precision were obtained for all peaks.

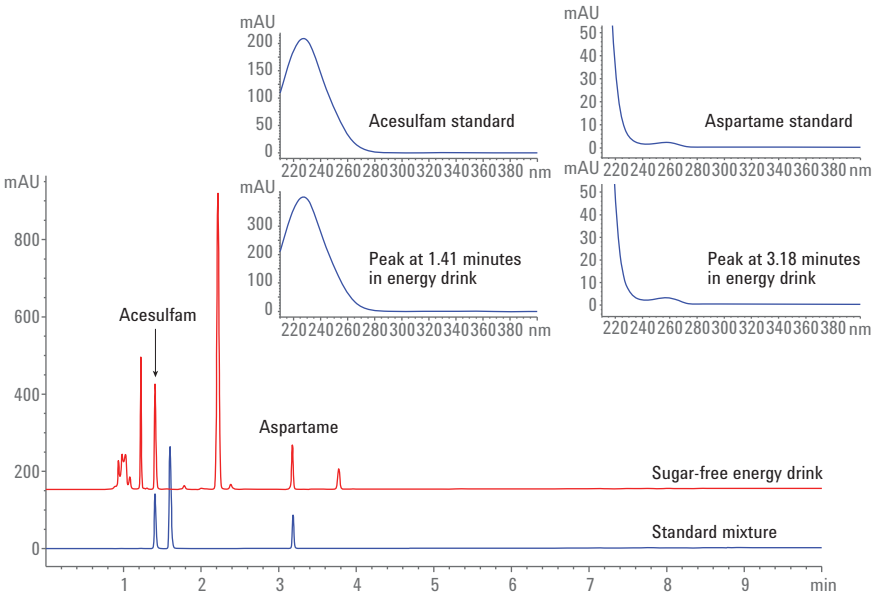
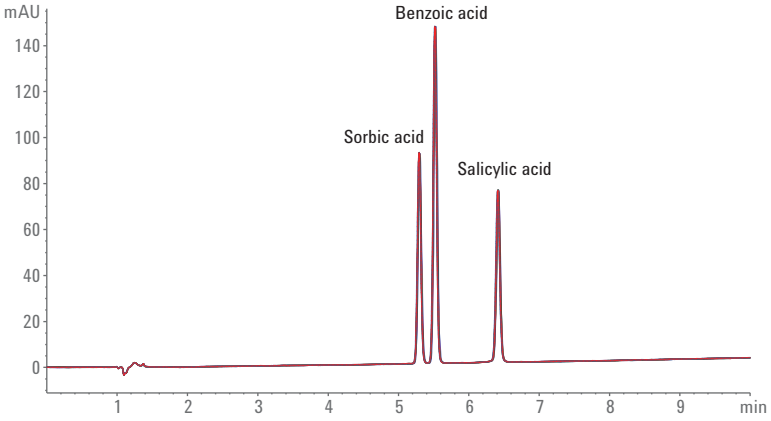


Figure 7. Analysis of sweeteners: Identification of acesulfam and aspartame in sugar-free energy drink.



Compound	RT (min)	RT RSD (%)	Area	Area RSD (%)	Resolution
Sorbic acid	5.30	0.08	319.2	0.03	–
Benzoic acid	5.52	0.08	515.6	0.04	2.4
Salicylic acid	6.42	0.07	293.8	0.18	9.2

Figure 8. Analysis of preservatives: Overlay of 10 consecutive analyses of the standard mixture and determination of retention time and area precision.

A facial tonic was analyzed as an example for the analysis of a real sample. In the facial tonic sample, salicylic acid could be identified based on the retention time and UV spectrum (Figure 9).

Conclusion

The Agilent 1260 Infinity II Multimethod solution supports running multiple LC applications with different columns and mobile phases on one LC system without manual system changes. This Application Note demonstrates the analysis of antioxidants, sweeteners, and preservatives as application examples from a food control laboratory. Excellent retention time and area precision were obtained for all application examples. Based on retention time and UV spectra, antioxidants, sweeteners, and preservatives could be identified in food and cosmetic samples.

References

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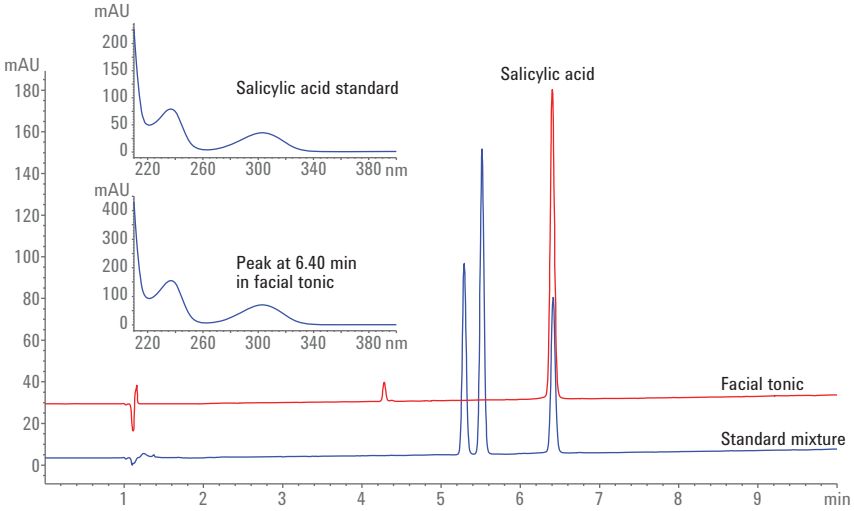

Figure 9. Analysis of preservatives: Identification of salicylic acid in facial tonic.

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