Automated Switching Between 1D-LC and Comprehensive 2D-LC Analysis – The Agilent 1290 Infinity 2D-LC Solution

Technical Overview

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Abstract

This Technical Overview shows a flexible setup of the Agilent 1290 Infinity 2D-LC Solution that allows switching between 1D-LC and comprehensive 2D-LC analysis with a few mouse clicks, and without any hardware change. This is accomplished by the installation of an additional 2-position/6-port valve. Using the determination of phenolic compounds in virgin olive oil as an example, it was shown that the additional 2-position/6-port valve does not have any negative impact on the separation, neither for 1D-LC analysis, nor for comprehensive 2D-LC analysis.

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1290 Infinity II LC

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Introduction

One dimensional liquid chromatography (1D-LC) is routinely applied to the analysis of all kinds of samples in different fields. However, it does not provide enough resolving power, or peak capacity, to resolve all components of complex samples[1]. Comprehensive two-dimensional liquid chromatography (comprehensive 2D-LC, LCxLC) can be employed to greatly increase the peak capacity compared to 1D-LC methods.

In LCxLC, the entire sample is subjected to separation in two dimensions. This is accomplished by alternately collecting the effluent from the first dimension column in two loop capillaries and injecting the collected effluent onto the second dimension column. When an orthogonal column configuration with noncorrelated separation mechanisms in the first and second dimension is used, the total peak capacity equals the product of the first and second dimension peak capacities[1,2]. In most implementations of LCxLC, some degree of correlation exists between the first and second dimension separation mechanisms, especially if two reversed phase separations are combined (RPxRP). In this case, only part of the available two-dimensional separation space is used, and the peaks are distributed, for example, inside of a triangular sector within the available separation space[3]. This reduces the resulting peak capacity. The accessible two-dimensional separation space can be enlarged by designing complex gradients for the second dimension separation, thereby increasing peak capacity.

Usually, it is necessary to reconnect capillaries to switch between performing 1D-LC methods and comprehensive 2D-LC methods using the same LC system. This Technical Overview shows the setup of the Agilent 1290 Infinity 2D-LC Solution using an additional 2-position/6-port valve to allow switching between 1D-LC and comprehensive 2D-LC analysis with a few mouse clicks, and without any hardware change.

Experimental

Equipment

The Agilent 1290 Infinity 2D-LC Solution was comprised of the following modules:

- Two Agilent 1290 Infinity Binary Pumps (G4220A)
- Agilent 1290 Infinity Autosampler (G4226A) with 1290 Infinity Thermostat (G1330B)
- Agilent 1290 Infinity Thermostatted Column Compartment (G1316C) with 2-position/6-port ultra high pressure valve head, 1,200 bar (p/n 5067-4117)
- Agilent 1290 Infinity Valve Drive (G1170A) with 2-position/4-port-duo valve (2D-LC Valve Head, 1,200 bar p/n 5067-4214) equipped with two 60-µL loops
- Agilent 1290 Infinity Diode Array Detector (G4212A) with a 60-mm Max-Light Cartridge Cell (G4212-60007)

Software

- Agilent OpenLAB CDS ChemStation Edition Rev. C.01.05 [38] with 1290 Infinity 2D-LC Acquisition Software Product Version A.01.01 [26].
- GC Image LCxLC Edition Software for 2D-LC data analysis from GC Image LLC., Lincoln, NE, USA.

Chemicals

All solvents were LC grade. Acetonitrile and methanol were 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, Billerica, MA, USA). Formic acid was purchased from Sigma-Aldrich, Steinheim, Germany.

Sample

Olive oil was purchased directly from an Italian olive oil farm, and sample preparation was carried out according to the protocol from the International Olive Council (COI/T.20/Doc No 29, November 2009)[4].

Setup

To allow switching between 1D-LC and comprehensive 2D-LC analysis without any hardware change, an additional 2-position/6-port valve was included in the setup of the Agilent 1290 Infinity 2D-LC Solution. During comprehensive 2D-LC analysis, a 2-position/4-port-duo valve was used for modulation, as shown in a previous Technical Overview[5]. The setup of the system and the plumbing of the 2-position/6-port valve, as well as the 2-position/4-port-duo valve are shown in Figures 1 and 2. In Figure 1, showing 1D-LC analysis, the 2-position/6-port valve is set to connect ports 1 and 2, directing the effluent from the first dimension column to the detector. The 2-position/4-port-duo valve does not switch during 1D-LC analysis, and the flow rate of the second dimension pump can be set to 0 mL/min. Alternatively, the second dimension column can be flushed during 1D-LC analysis.

In Figure 2, showing comprehensive 2D-LC analysis, the 2-position/6-port valve is set to connect ports 1 and 6, directing the effluent from the first dimension column to the 2-position/4-port-duo valve. Using the 2-position/4-port-duo valve, the effluent from the first dimension column is alternately collected in two loops and injected onto the second dimension column.

The 2-position/6-port valve used to switch between 1D-LC and comprehensive 2D-LC analysis was installed in the Agilent 1290 Infinity Thermostatted Column Compartment, and the 2-position/4-port-duo valve used for modulation was operated by an Agilent 1290 Infinity Valve Drive.

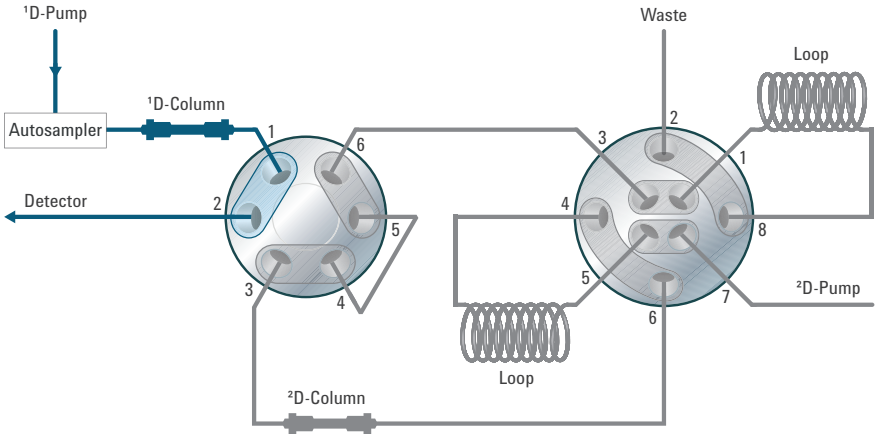

Figure 1. Setup of the Agilent 1290 Infinity 2D-LC Solution with an additional 2-position/6-port valve positioned for 1D-LC analysis.

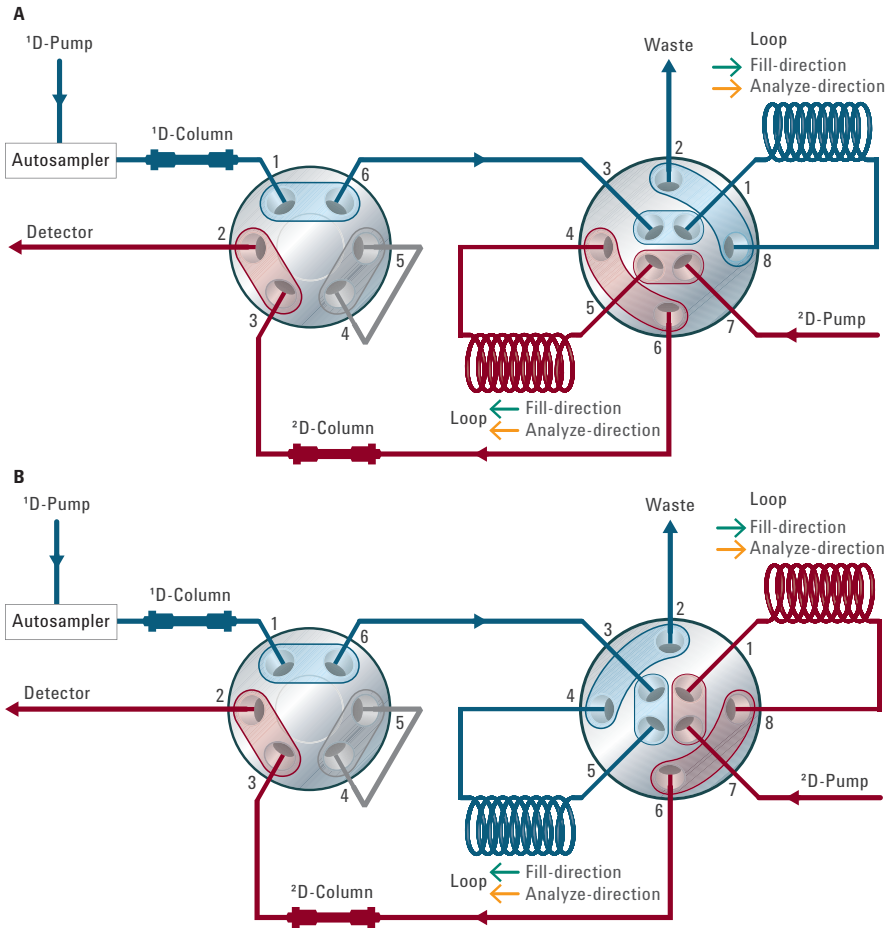

Figure 2. Setup of the Agilent 1290 Infinity 2D-LC Solution with an additional 2-position/6-port valve positioned for comprehensive 2D-LC analysis; (A) Collection of the effluent from the first dimension column in loop 1 (between ports 1 and 8); (B) Collection of the effluent from the first dimension column in loop 2 (between ports 4 and 5), analysis of the content of loop 1 on the second dimension column.

To switch between 1D-LC and comprehensive 2D-LC analysis, respective methods with the appropriate setting of the 2-position/6-port valve have to be defined. In the instrument configuration, 2D-LC has to be disabled for 1D-LC analysis and enabled for comprehensive 2D-LC analysis. This can be done with one mouse click, as shown in Figure 3.

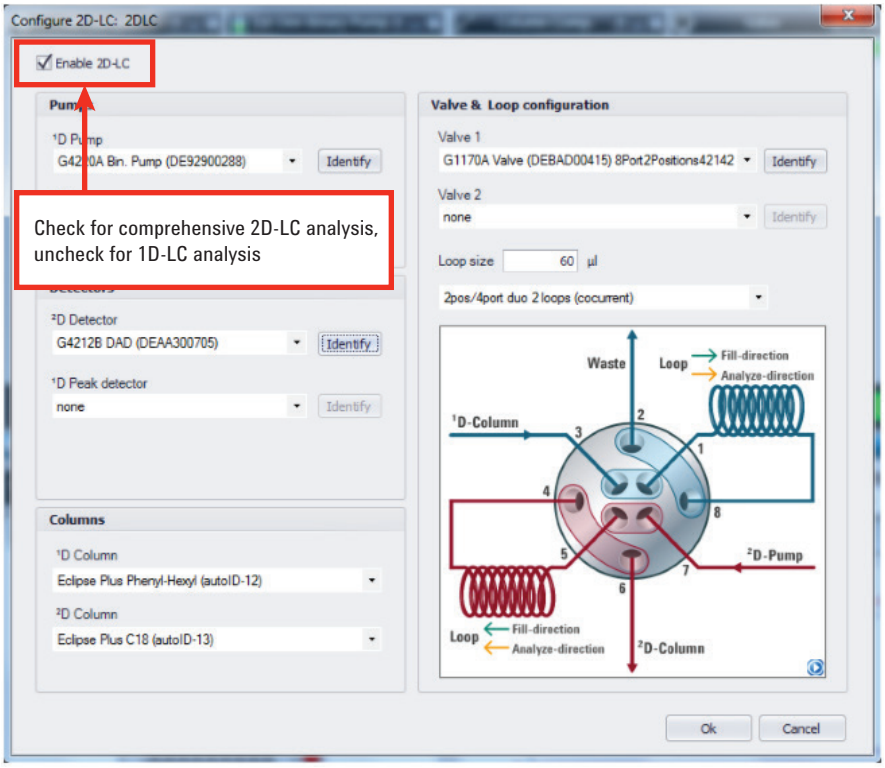

Figure 3. Enabling and disabling of 2D-LC in the instrument configuration.

1D-LC method

Column	
Agilent ZORBAX RRHD Eclipse Plus Phenyl-Hexyl, 2.1 × 150 mm, 1.8 µm (p/n 959759-912)	
2-position/6-port valve	
The 2-position/6-port valve was set to connect ports 1 and 2 to direct the effluent from the column to the detector (Figure 1).	
Pump	
Solvent A	Water + 0.1 % formic acid
Solvent B	Methanol + 0.1 % formic acid
Flow rate	0.2 mL/min
Gradient	0 minutes – 5 % B 15 minutes – 95 % B 20 minutes – 95 % B
Stop time	20 minutes
Post time	8 minutes
Thermostatted Column Compartment	
Column temperature	Maintained at 25 °C
Autosampler	
Injection volume	5 µL
Sample temperature	6 °C
Needle wash	6 seconds in methanol
Diode Array Detector	
Wavelength	260 nm/4 nm, Ref.: 360 nm/100 nm
Data rate	20 Hz

Comprehensive 2D-LC method

Columns	
First dimension	Agilent ZORBAX RRHD Eclipse Plus Phenyl-Hexyl, 2.1 × 150 mm, 1.8 µm (p/n 959759-912)
Second dimension	Agilent ZORBAX RRHD Eclipse Plus C18, 3.0 × 50 mm, 1.8 µm (p/n 959757-302)
2-position/6-port valve	
The 2-position/6-port valve was set to connect ports 1 and 6 to direct the effluent from the first dimension column to the 2-position/4-port-duo valve (Figure 2).	
First dimension pump	
Solvent A	Water + 0.1 % formic acid
Solvent B	Methanol + 0.1 % formic acid
Flow rate	0.05 mL/min
Gradient	0 minutes – 5 % B 60 minutes – 95 % B 80 minutes – 95 % B
Stop time	80 minutes
Post time	30 minutes
Second dimension pump	
Solvent A	Water + 0.1 % formic acid
Solvent B	Acetonitrile + 0.1 % formic acid
Flow rate	3 mL/min
Gradient	0.00 minutes – 5 % B 0.35 minutes – 15 % B 0.36 minutes – 5 % B 0.50 minutes – 5 % B
Gradient modulation	0.00 min – 5 % B to 15 minutes – 5 % B to 60 minutes – 35 % B to 65 minutes – 65 % B 0.35 minutes – 15 % B to 15 minutes – 15 % B to 60 minutes – 60 % B to 65 minutes – 95 % B 0.36 minutes – 5 % B to 15 minutes – 5 % B to 60 minutes – 35 % B to 65 minutes – 65 % B 0.50 minutes – 5 % B to 15 minutes – 5 % B to 60 minutes – 35 % B to 65 minutes – 65 % B
Post time	30 minutes
Thermostatted Column Compartment	
First dimension column on the right side at 25 °C	
Second dimension column on the left side at 60 °C	
2-position/4-port-duo valve	
The 2-position/4-port-duo valve was switched automatically after each second dimension modulation cycle of 30 seconds. The loops were used in a cocurrent manner (filled and eluted from the same side).	
Autosampler	
Injection volume	20 µL
Sample temperature	6 °C
Needle wash	6 seconds in methanol
Diode Array Detector	
Wavelength	260 nm/4 nm, Ref.: 360 nm/100 nm
Data rate	80 Hz

The method setup for the second dimension pump with the complex gradient designed to enlarge the accessible two-dimensional separation space is shown in Figure 4.

Results and Discussion

This Technical Overview shows the setup of the Agilent 1290 Infinity 2D-LC Solution using an additional 2-position/6-port valve to allow switching between 1D-LC and comprehensive 2D-LC analysis. In 1D-LC analysis, the additional 2-position/6-port valve is installed in the flow path behind the column. In comprehensive 2D-LC analysis, respectively, the valve is installed behind the first dimension column as well as the second dimension column. To demonstrate the performance of this setup, it is shown that the additional 2-position/6-port valve does not have any negative impact on the separation, neither for 1D-LC analysis, nor for comprehensive 2D-LC analysis.

The determination of phenolic compounds in virgin olive oil using comprehensive 2D-LC shown in a previous Application Note[6] was chosen to demonstrate the performance of the setup. For comprehensive 2D-LC analysis, the method described in that Application Note[6] was used. For 1D-LC analysis, the flow rate and gradient time for the (first dimension) column were changed to typical values used in 1D-LC analysis. Both the 1D-LC analysis and the comprehensive 2D-LC analysis, were performed using an exemplary olive oil with the additional 2-position/6-port valve in the flow path, and without this valve for comparison.

Figure 5 shows an overlay of six 1D-LC analyses of the exemplary olive oil, three analyses performed with the additional 2-position/6-port valve in the flow path and three analyses performed without this valve. No difference between the analyses performed with and without the additional 2-position/6-port valve in the flow path can be observed.

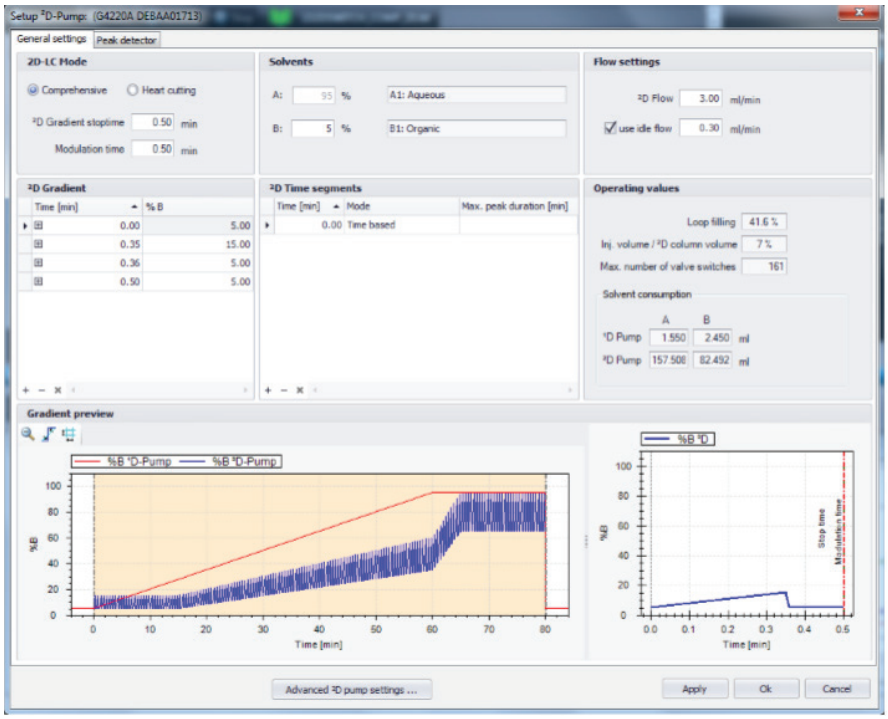

Figure 4. Method setup for the second dimension pump.

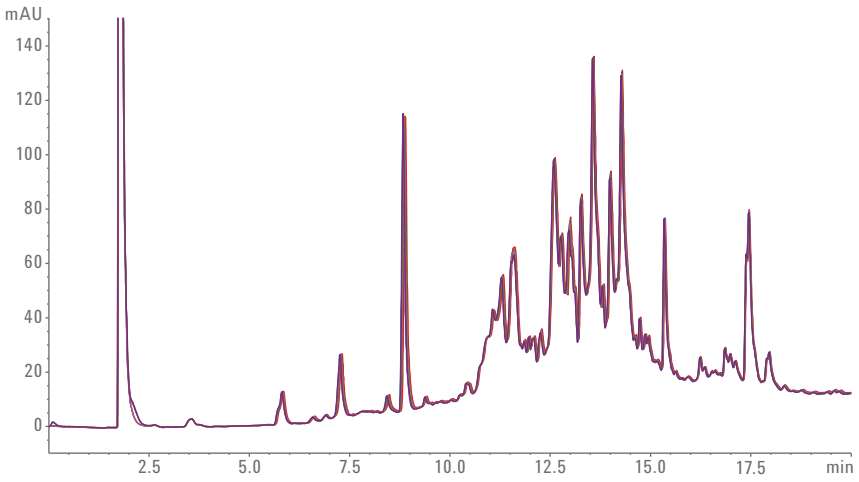

Figure 5. Overlay of six 1D-LC analyses of an olive oil, three analyses performed with the additional 2-position/6-port valve in the flow path and three analyses performed without this valve.

Figure 6 shows two comprehensive 2D-LC analyses of the exemplary olive oil. One analysis was done with the additional 2-position/6-port valve in the flow path (Figure 6A) and one analysis was done without this valve (Figure 6B). As observed for 1D-LC analysis, no impact of the additional 2-position/6-port valve can be seen during comprehensive 2D-LC analysis. The only difference between the two comprehensive 2D-LC analyses shown in Figure 6 is the reduced second dimension retention time of peaks eluting in the first dimension retention time range from 60 to 65 minutes, in Figure 6B (for example, see highlighted peak). This can be explained by the fact that these peaks were detected one modulation cycle later in Figure 6B compared to Figure 6A. The rapid modulation of the second dimension gradient in the time range from 60 to 65 minutes causes the observed retention time differences.

Conclusion

This Technical Overview shows the setup of the Agilent 1290 Infinity 2D-LC Solution comprising an additional 2-position/6-port valve to allow switching between 1D-LC and comprehensive 2D-LC analysis with a few mouse clicks, and without any hardware change. Using the determination of phenolic compounds in virgin olive oil, it is shown that the additional 2-position/6-port valve has no negative impact on the separation, neither for 1D-LC analysis, nor for comprehensive 2D-LC analysis. Therefore, the additional 2-position/6-port valve allows a flexible setup of the Agilent 1290 Infinity 2D-LC Solution for 1D-LC and comprehensive 2D-LC analysis.

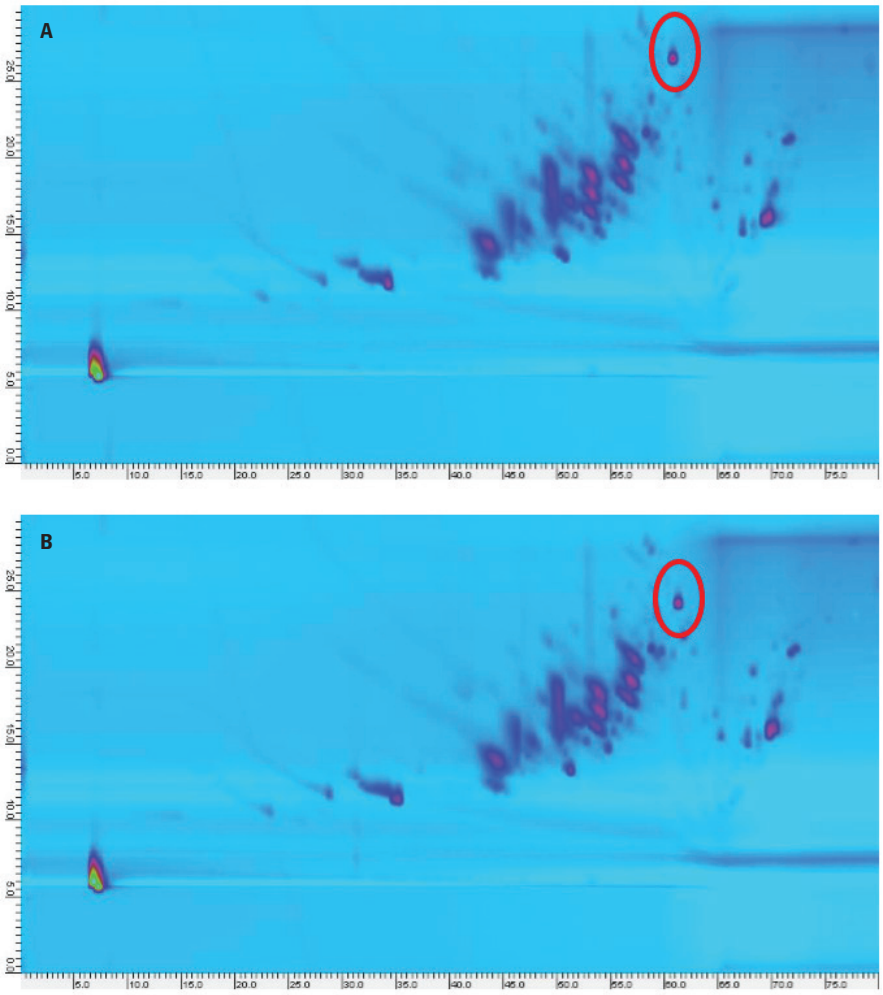

Figure 6. Comprehensive 2D-LC analysis of an olive oil; A) performed with the additional 2-position/6-port valve in the flow path; B) performed without the additional 2-position/6-port valve.

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