Application of Multiple Heart-Cutting 2D-LC in Method Development for Impurity Analysis

The Agilent 1290 Infinity 2D-LC Solution

Application Note

Small Molecule Pharmaceuticals and Generics

Author

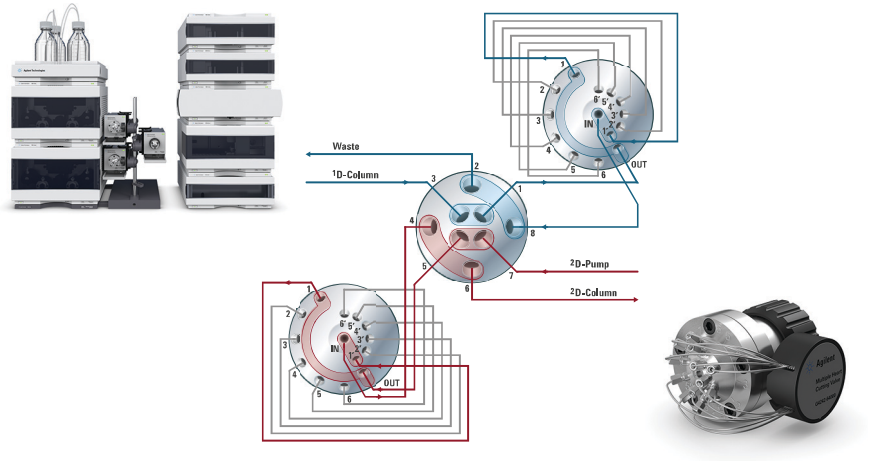
Sonja Krieger

Agilent Technologies, Inc.

Waldbronn, Germany

Abstract

The analysis of impurities is of great importance during the development and production of pharmaceuticals and fine chemicals. This Application Note shows that multiple heart-cutting 2D-LC is ideally suited for method development for impurity analysis of pharmaceuticals and fine chemicals. Using the Agilent 1290 Infinity 2D-LC Solution, every peak detected after the first-dimension separation was heart-cut and transferred to a second-dimension separation with different selectivity. This approach enabled the discovery of possible coeluting compounds. In addition, the identity of impurities could be confirmed by spiking the sample with the suspected impurity.



Agilent Technologies

Introduction

The analysis of impurities in pharmaceuticals and fine chemicals is of great importance in the pharmaceutical and chemical industries. For new drug substances, ICH guideline Q3A (R2) mandates that impurities present at or above 0.05 % must be reported, and that impurities at or above 0.1 % must be identified[1].

The impurity analysis of pharmaceuticals and fine chemicals can be realized by analyzing a concentrated solution of the substance using liquid chromatography. Impurities in pharmaceutical substances or fine chemicals often show structural similarity to the main compound and to each other. Therefore, it might be impossible to achieve separation using a system with a given selectivity (column-solvent combination). One solution to this problem is heart-cutting of coeluting compounds and transfer to a separation system with different selectivity, thereby enabling separation. The capability to resolve an impurity that coelutes with the main compound using heart-cutting 2D-LC with the Agilent 1290 Infinity 2D-LC Solution was shown in a previous Application Note[2].

During method development for impurity analysis, all impurities must be detected, and no impurity must be hidden due to coelution with the main compound or another impurity. This can be ensured using heart-cutting 2D-LC. The 1290 Infinity 2D-LC Solution enables heart-cutting of multiple peaks from the first-dimension separation, storage of the heart-cuts in loops, and sequential second-dimension analysis of the stored heart-cuts.

This Application Note demonstrates the multiple heart-cutting 2D-LC analysis of a sample representing a pharmaceutical compound. Every peak detected after the first-dimension separation was subjected to heart-cutting and analysis in a second-dimension separation with different selectivity to enable the discovery of possible coelution in the first-dimension separation.

Experimental

2D-LC method

First-dimension pump	
Solvent A	Water + 0.1 % formic acid
Solvent B	Acetonitrile + 0.1 % formic acid
Flow rate	0.2 mL/min
Gradient	5 %B at 0 minutes 95 %B at 20 minutes 95 %B at 25 minutes
Post time	10 minutes
Second-dimension pump	
Solvent A	Water + 0.1 % formic acid
Solvent B	Methanol + 0.1 % formic acid
Flow rate	2.0 mL/min
Gradient and gradient modulation	10 %B at 0.00 minutes to 50 %B at 22.00 minutes 70 %B at 1.50 minutes to 95 %B at 22.00 minutes
^{2}D gradient stop time	1.50 minutes
^{2}D cycle time	2.00 minutes
Autosampler	
Injection volume	2 µL
Sample temperature	6 °C
Needle wash	10 seconds in methanol/water (50/50, v/v)
Thermostatted column compartment	
First-dimension column	30 °C at right side
Second-dimension column	30 °C at left side
Multiple heart-cutting	
Operation mode	• Time-based multiple heart-cutting was performed with the heart-cuts (2D time segments) set according to the first-dimension retention times. • Heart-cutting of impurities was conducted with a sampling time of 0.40 minutes (loop filling of 200 %). • Three closely spaced consecutive heart-cuts were taken across the main compound's peak using a sampling time of 0.10 minutes (loop filling of 50 %).
Diode array detection	
First dimension	Wavelength 254 nm/4 nm, reference 360 nm/100 nm 20-Hz data rate
Second dimension	Wavelength 254 nm/4 nm, reference 360 nm/100 nm 80-Hz data rate

Instrumentation

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

- Agilent 1290 Infinity Binary Pump (G4220A, 2x)
- Agilent 1290 Infinity Autosampler (G4226A) with Agilent 1290 Infinity Thermostat (G1330B)
- Agilent 1290 Infinity Thermostatted Column Compartment (G1316C)
- Agilent 1290 Infinity Valve Drive (G1170A) with 2-position/4-port duo-valve (2D-LC valve head, G4236A)
- Agilent 1290 Infinity Valve Drive (G1170A, 2x) with multiple heart-cutting valves (G4242-64000, 2x) equipped with 40-µL loops
- Agilent 1290 Infinity Diode Array Detector (G4212A, 2x) with 10-mm Max-Light cartridge cell (G4212-60008)

Software

Agilent OpenLab CDS ChemStation Edition Software, version C.01.07 [27] with Agilent 1290 Infinity 2D-LC Acquisition Software, version A.01.02 [24]

Columns

- First dimension: Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 × 150 mm, 1.8 µm (p/n 959759-902)
- Second dimension: Agilent Poroshell 120 Bonus-RP, 4.6 × 50 mm, 2.7 µm (p/n 699968-901)

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 from Agilent (p/n G2453-85060).

Sample

As sample representing a pharmaceutical substance containing a range of impurities, a solution of N,N-diethyl-*m*-toluamide (DEET) in methanol/water (50/50, v/v) with several added impurities was used.

Results and Discussion

An important question that arises during method development for impurity analysis of pharmaceuticals or fine chemicals is whether all impurities are separated from the main compound and from each other using a given method, or, whether coelution occurs. Figure 1 shows a one-dimensional impurity analysis using a C18-column with a water-acetonitrile gradient, each with 0.1 % formic acid. In the enlarged chromatogram, partial coelution can be observed for the main compound and two impurities. To resolve this partial coelution and inspect every peak for possible coelution, heart-cutting of the peaks and transfer to a second-dimension separation with different selectivity can be deployed.

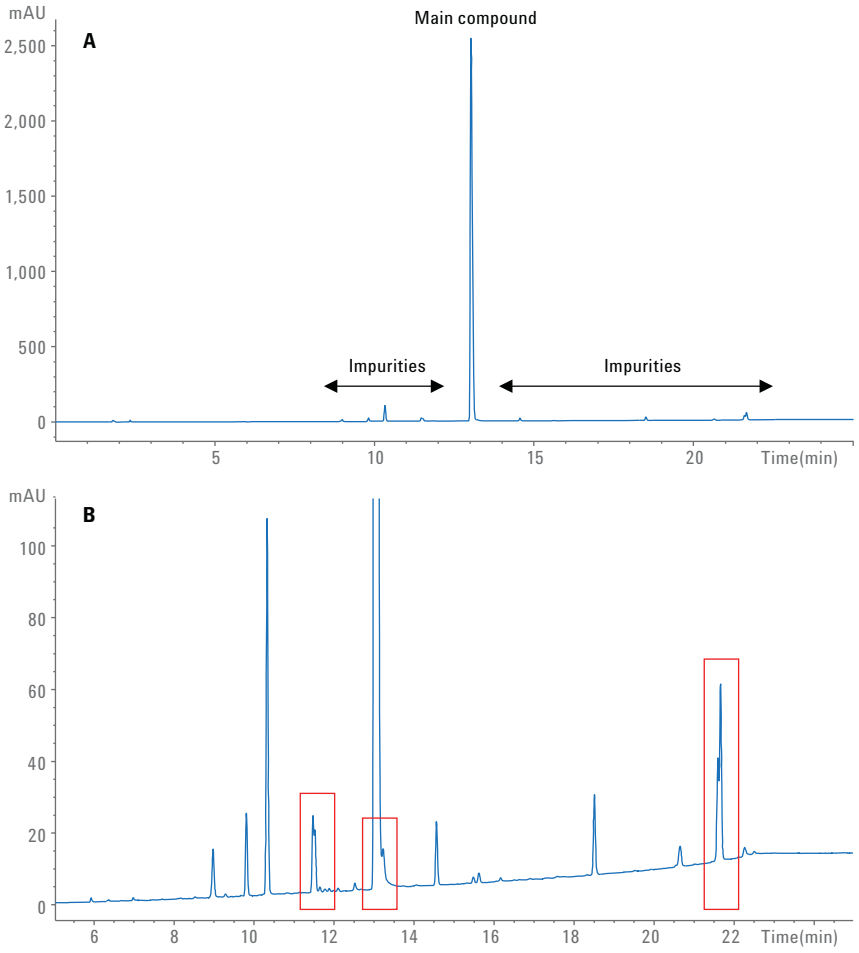


Figure 1. One-dimensional impurity analysis according to the first-dimension conditions of the described 2D-LC method; partial coelution is marked; (A) full-scale; (B) magnified.

Panel A in Figure 2 shows the plumbing diagram for multiple heart-cutting with the central 2D-LC valve in cocurrent configuration and two multiple heart-cutting valves. Panel B in Figure 2 shows a multiple heart-cutting valve with pre-installed, pressure-tested 40-µL loops.

The multiple heart-cutting algorithm ensures that valve switching is reduced to a minimum, and that heart-cuts taken from the first-dimension effluent are analyzed in the second dimension as quickly as possible. For every multiple heart-cutting 2D-LC analysis, the first heart-cut is taken and held in loop 1 of deck A (multiple heart-cutting valve on the right in Figure 2A). The central 2D-LC valve then switches, and the first heart-cut is analyzed in the second dimension. During the second-dimension analysis of the first heart-cut, up to five heart-cuts can be taken and held in loops 1 to 5 of deck B (multiple heart-cutting valve on the left in Figure 2A) and stored until their analysis in the second dimension. In each deck (multiple heart-cutting valve), one loop is always required for flow-through of first-dimension effluent and second-dimension solvent respectively. When the second-dimension analysis of the first heart-cut is complete and the central 2D-LC valve switches again, up to five heart-cuts can be stored in loops 1 to 5 of deck A, while the heart-cuts stored in deck B are analyzed in the second dimension. When storing heart-cuts in a deck, a flush gradient is performed before analysis of the heart-cuts stored in this deck and the second-dimension analysis of stored heart-cuts is done in reversed storage order.

The multiple heart-cutting algorithm enables starting the second-dimension analysis of heart-cuts while the first-dimension analysis is still running. This approach means that loops can be reused for heart-cutting during the same analysis and more heart-cuts can be taken than the number of loops fitted. A more detailed description of the multiple heart-cutting algorithm, the operational routine, and software features can be found in a Technical Overview[3].

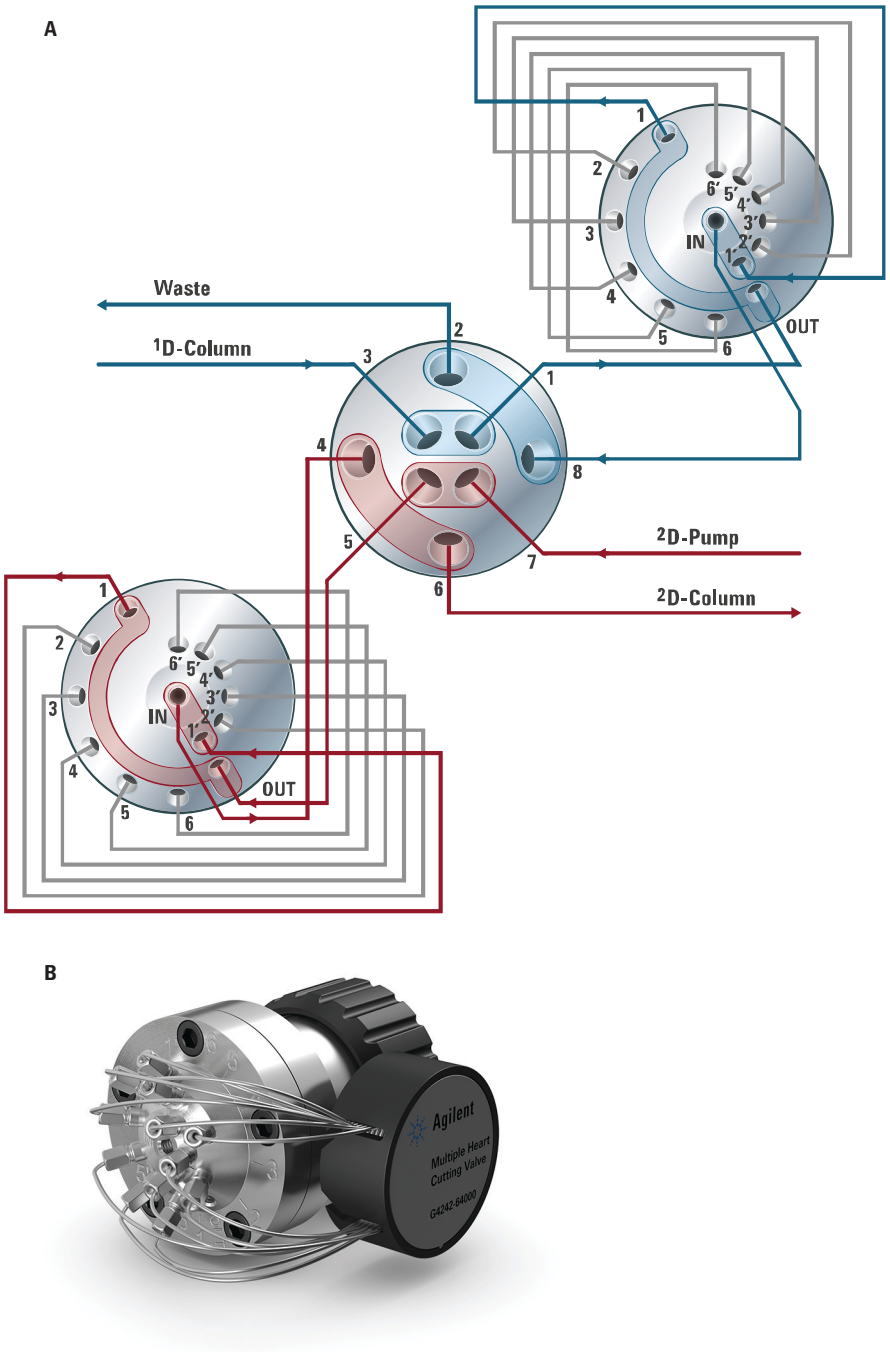


Figure 2. A) Plumbing diagram for multiple heart-cutting with the central 2D-LC valve in cocurrent configuration and two multiple heart-cutting valves. B) Multiple heart-cutting valve with pre-installed loop capillaries.

Multiple heart-cutting can be performed time-based (with dedicated heart-cut times, for example, for known first-dimension separations) or peak-based (with heart-cutting being triggered by detection of a first-dimension peak, for example, for unknown samples). For the impurity analysis shown in Figure 1, time-based multiple heart-cutting was used. To enable the discovery of coeluting sample components, every impurity was selected for heart-cutting and analyzed in the second dimension. In addition, three closely spaced consecutive heart-cuts were performed across the peak originating from the main compound.

Figure 3 shows the method setup for multiple heart-cutting. In the gradient preview section, a first-dimension reference chromatogram can be loaded and time segments for heart-cutting can be automatically generated. The generated time segments can be edited in the ^{2}D time segments table and additional heart-cuts can be added manually to investigate an interesting region more closely. For heart-cutting of the impurities, a sampling time of 0.40 minutes was chosen, which corresponded to a loop filling of 200 % (40-µL loop size, 0.20-mL/min first-dimension flow rate). Due to loop overfill, the loops were entirely filled with first-dimension effluent. The enlarged part of the gradient preview section shows the time segments for heart-cutting of the first four impurities. The orange area represents the sampling time with the darker shaded area predicting the part of the first-dimension effluent that is transferred to the second dimension. For performing three closely spaced consecutive heart-cuts across the main compound's peak, a sampling time of 0.10 minutes (50 % loop filling) was chosen. This way, the first-dimension effluent containing the main compound's peak was sampled without any loss.

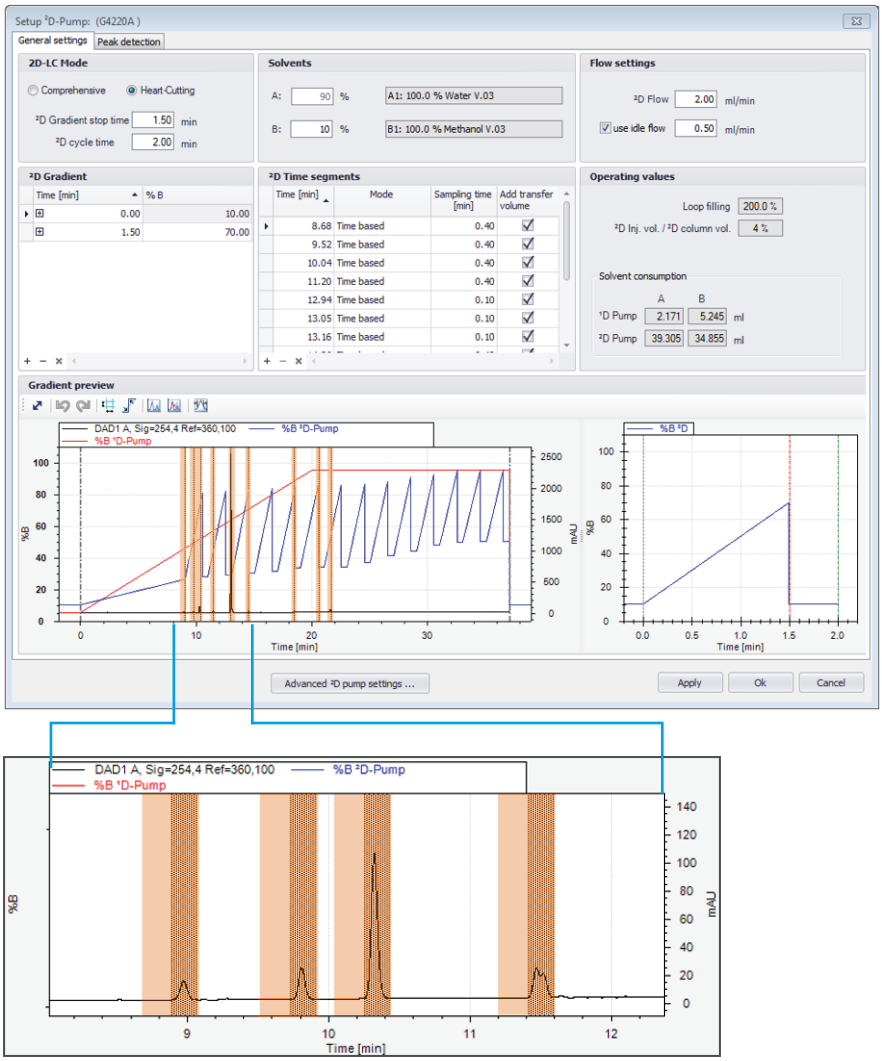


Figure 3. Method setup for multiple heart-cutting.

Figure 4 shows the results of the multiple heart-cutting 2D-LC analysis presented in the 2D-LC heart-cut viewer. In the top two panels, the first-dimension chromatogram (left) with the marked heart-cuts and the heart-cut table (right) is shown. At the bottom, the second-dimension chromatogram as recorded by the second-dimension detector is shown on the left. Here, the order of second-dimension analyses of the heart-cuts with analysis in reversed storage order and flush gradients (F) can be retraced. On the right, single second-dimension chromatograms can be displayed and overlaid.

The results obtained from the second-dimension analyses of the heart-cuts from the impurities are displayed in Figure 5. For heart-cuts number 4 and 11, compounds that partially coeluted in the first dimension could be separated in the second dimension.

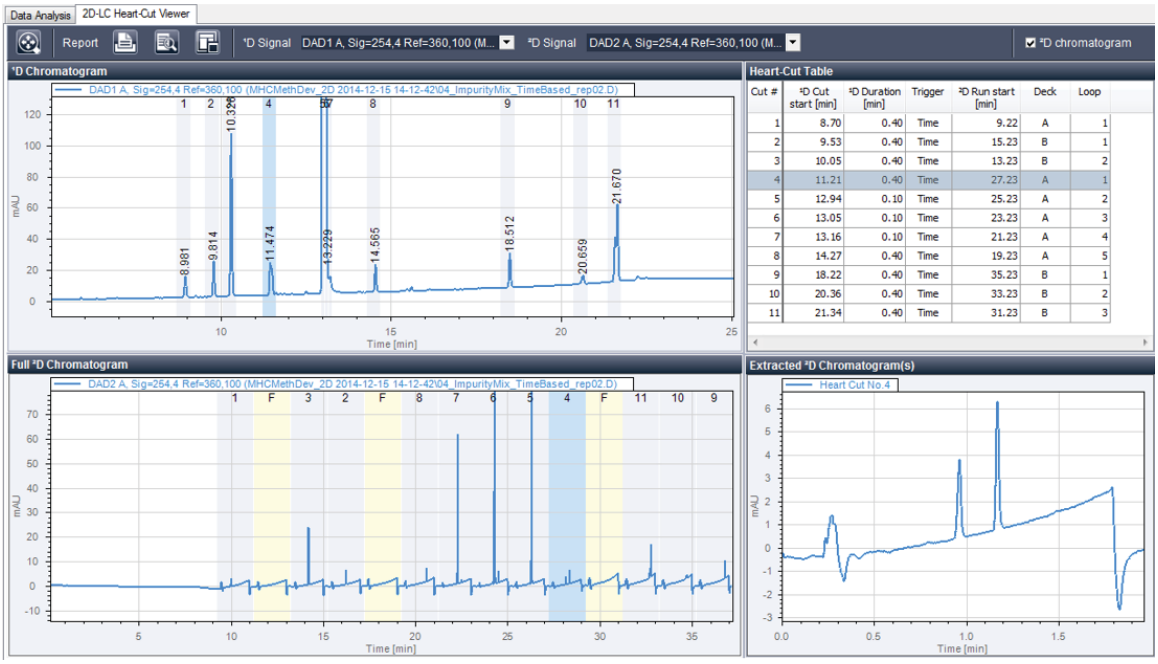


Figure 4. Results of the multiple heart-cutting 2D-LC analysis presented in the 2D-LC heart-cut viewer.

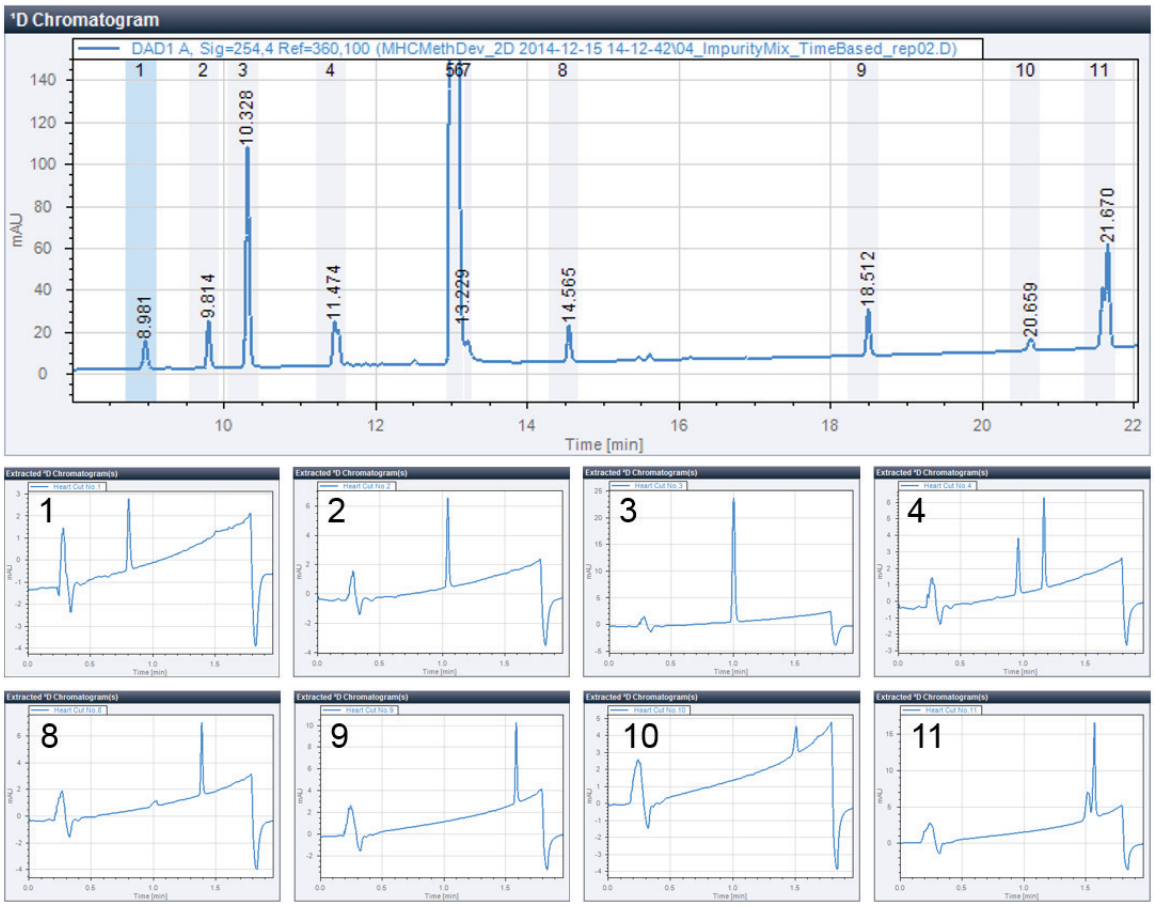


Figure 5. Results of the second-dimension analyses of the impurities. Upper panel: first-dimension chromatogram with marked heart-cuts. Lower panels: second-dimension chromatograms.

Figure 6 shows the results obtained from the second-dimension analyses of the three closely spaced consecutive heart-cuts taken across the main compound's peak. Overall, the second-dimension analyses revealed coelution of three impurities with the main compound. One impurity can be detected across the whole main compound's peak (in heart-cuts 5 to 7). A second impurity appears in heart-cuts 6 and 7 and a third impurity that partially coelutes with the main compound in the first dimension is separated in heart-cut 7.

To confirm the identity of an impurity, the suspected compound can be synthesized and spiked in the sample during the impurity analysis. As an example, the sample solution was spiked with an impurity that coeluted with the main compound in the first dimension and was separated from the main compound in the second dimension. Figure 7 shows the analysis of the sample spiked with increasing amounts of the impurity. After heart-cutting, the increasing amounts of the impurity could be detected during the second-dimension analysis. Multiple heart-cutting 2D-LC offers the linearity and repeatability necessary for reliable quantification, as described in a Technical Overview[3].

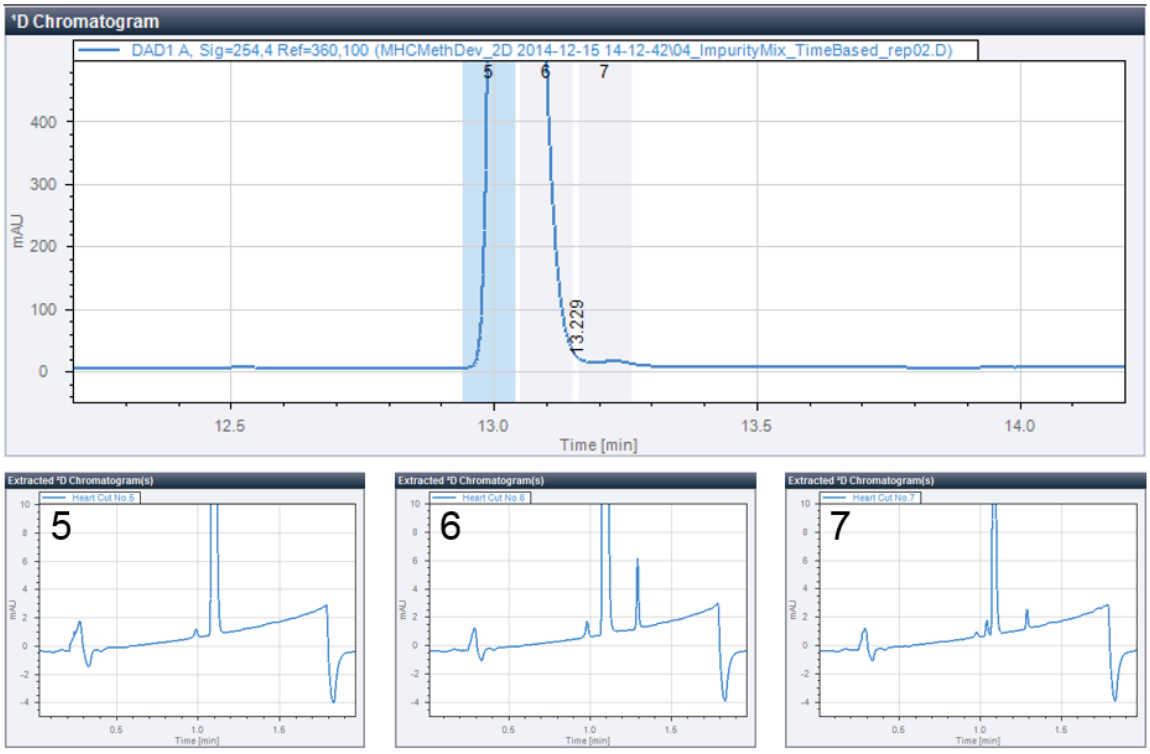


Figure 6. Results of the second-dimension analyses of the main compound. Upper panel: first-dimension chromatogram with marked heart-cuts. Lower panels: second-dimension chromatograms.

Conclusion

The Agilent 1290 Infinity 2D-LC Solution with multiple heart-cutting enables heart-cutting of multiple peaks from a first-dimension separation and transfer to a second-dimension separation with different selectivity. This Application Note shows that multiple heart-cutting 2D-LC is ideally suited for method development for impurity analysis of pharmaceutical substances, fine chemicals, or pharmaceutical and chemical formulations. Transfer of peaks detected after a first-dimension separation to a second-dimension separation enables discovery of possible coelution.

References

1. International conference on harmonization of technical requirements for registration of pharmaceuticals for human use, ICH harmonized tripartite guideline, Impurities in new drug substances (Q3A(R2)), October 25, **2006**.

2. Naegele, E., Detection of Impurities by Heart-Cutting using the Agilent 1290 Infinity 2D-LC Solution, *Agilent Technologies Application Note*, publication number 5991-0834EN, **2012**.

3. Buckenmaier, S., Multiple Heart-Cutting with the Agilent 1290 Infinity 2D-LC Solution, *Agilent Technologies Technical Overview*, publication number 5991-5615EN, **2015**.

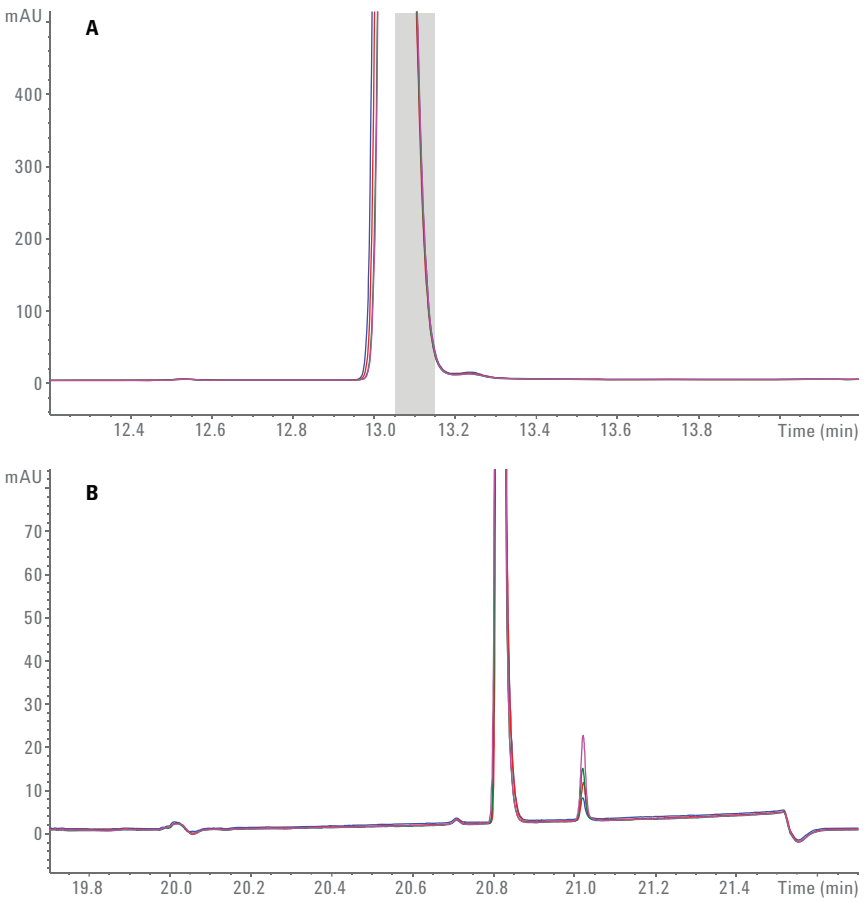


Figure 7. Results of the analysis of the sample spiked with increasing amounts (twice, three-times, and four-times the initial amount) of an impurity. A) Overlay of first-dimension chromatograms with marked heart-cut. B) Overlay of second-dimension chromatograms.

www.agilent.com/chem

Published in the USA, June 1, 2015
5991-5643EN

Agilent Technologies