Achiral-Chiral Heart-Cutting 2D-LC Analysis of Chiral Pharmaceutical Substances

Impurity Analysis and Simultaneous Determination of Enantiomeric Composition Using the Agilent 1290 Infinity 2D-LC Solution

Application Note

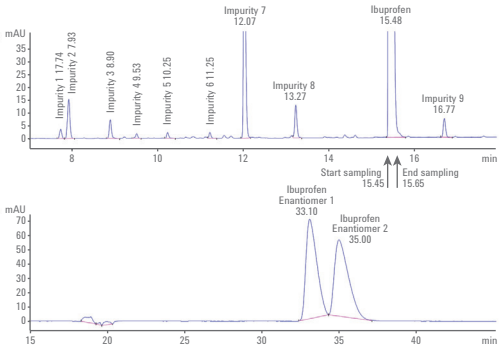
Small Molecule Pharmaceuticals & Generics

Author

Sonja Krieger
Agilent Technologies, Inc.
Waldbronn, Germany

Abstract

This Application Note demonstrates that the Agilent 1290 Infinity 2D-LC Solution is ideally suited for the analysis of impurities in chiral pharmaceutical substances and the simultaneous determination of the enantiomeric composition of the active pharmaceutical ingredient (API). In the first dimension, a reversed phase separation is used to separate achiral impurities from the API. The API is transferred to a chiral second dimension column for determination of the enantiomeric composition in a heart-cutting experiment. The reliability of the heart-cutting process is shown using racemic ibuprofen as sample. In addition, a mixture containing R-(+)-thalidomide as main compound and S-(–)-thalidomide as trace compound is analyzed, and the enantiomeric excess is determined.



Verified for Agilent
1290 Infinity II LC

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Introduction

The analysis of impurities in drugs is critical in the pharmaceutical industry. According to ICH guideline Q3A (R2), impurities at or above 0.05 % in new drug substances must be reported, and impurities at or above 0.1 % in new drug substances must be identified[1]. Enantiomeric impurities are excluded from this guideline.

Enantiomers of chiral drugs often show differences in pharmacokinetic behavior and pharmacological activity. One enantiomer might be pharmacologically active, while the other might be pharmacologically inactive, or even toxic. Therefore, the United States Food and Drug Administration has released guidances on the development of new stereoisomeric drugs, demanding that the stereoisomeric composition of a drug with a chiral center is known, and that specifications for the final product include assurance of purity from a stereochemical viewpoint[2].

The most widely used technique to achieve enantiomer separation is liquid chromatography using a chiral stationary phase[3-5]. Chiral stationary phases possess limited chemical selectivity. For this reason, mixtures of several pairs of enantiomers can rarely be analyzed in one chromatographic run[6]. This precludes the simultaneous assessment of impurities in chiral drugs, and the determination of the enantiomeric composition of the API in one chromatographic run, using a chiral stationary phase.

This Application Note demonstrates the use of the Agilent 1290 Infinity 2D-LC Solution for the analysis of impurities in chiral drugs, and the simultaneous determination of the enantiomeric composition of the API in a heart-cutting experiment. In the first dimension, a reversed phase column is used to separate achiral impurities from the chiral API (for example, ibuprofen or thalidomide). In a heart-cutting experiment, the chromatographic peak of the API eluting from the first dimension column is transferred to the second dimension column. In the second dimension, a chiral stationary phase is used to enable separation of the enantiomers.

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)
- Agilent 1290 Infinity Valve Drive (G1170A) with a 2-Position/4-Port-Duo valve (G4236A) equipped with one 60-µL loop
- Two Agilent 1290 Infinity Diode Array Detectors (G4212A) with a 10-mm Max-Light Cartridge Cell (G4212-60008) and a 60-mm Max-Light Cartridge Cell (G4212-60007)

Columns

First dimension

Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 × 150 mm, 1.8 µm (p/n 959759-902)

Second dimension

Chiral column, 4.6 × 250 mm, 5 µm

Software

Agilent OpenLAB CDS A.02.01 (ChemStation Edition) with Agilent 1290 Infinity 2D-LC Acquisition Software Product Version A.01.01 [26]

Chemicals

Ibuprofen sodium salt (racemic), RS-(±)-thalidomide, R-(+)-thalidomide, and S(–)-thalidomide were purchased from Sigma-Aldrich, Steinheim, Germany.

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 and acetic acid were purchased from Sigma-Aldrich, Steinheim, Germany.

A stock solution of ibuprofen sodium salt was prepared at 10 mg/mL in water.

Dilutions of the stock solution (0.1, 0.2, 0.5, 0.75, and 1 mg/mL) were made with acetonitrile/water (30/70; v/v) + 0.1 % formic acid.

R-(+)-thalidomide and S(–)-thalidomide were dissolved in acetonitrile at 1.0 mg/mL.

A mixture containing R-(+)-thalidomide as the main compound, and S(–)-thalidomide as the trace compound was prepared from 100 µL of 1.0 mg/mL R-(+)-thalidomide, 1 µL of 1.0 mg/mL S-(–)-thalidomide, and 899 µL of acetonitrile/water (30/70; v/v) + 0.1 % acetic acid.

For degradation, a solution of 1.2 mg/mL RS-(±)-thalidomide in acetonitrile/water (50/50; v/v) + 1 % 1N NaOH was heated to 60 °C for 2 hours. To show the separation of degradation products from the main compound thalidomide, 10 µL of the degraded solution were added to the mixture containing R-(+)-thalidomide as the main compound and S(–)-thalidomide as the trace compound.

Method

First dimension pump, Agilent 1290 Infinity Binary Pump		
Solvent	Ibuprofen	A) Water + 0.1 % formic acid B) Acetonitrile + 0.1 % formic acid
	Thalidomide	A) Water + 0.1 % acetic acid B) Acetonitrile + 0.1 % acetic acid
Gradient	0 minutes – 5 % B 20 minutes – 95 % B 25 minutes – 95 % B 25.1 minutes – 5 % B	
Flow rate	0.25 mL/min	
Stop time	Ibuprofen: 45 minutes Thalidomide: 30 minutes	
Second dimension pump, Agilent 1290 Infinity Binary Pump		
Solvent	Ibuprofen	Water/Methanol (35/65) + 0.1 % formic acid
	Thalidomide	Methanol + 0.1 % acetic acid
Flow rate	1 mL/min	
Agilent 1290 Infinity Thermostatted Column Compartment		
First and second dimension column at 25 °C		
Agilent 1290 Infinity Autosampler		
Injection volume	Ibuprofen: 20 µL Thalidomide: 10 µL	
Sample temperature	6 °C	
Needle wash	6 seconds in methanol	
First dimension detector, Agilent 1290 Infinity Diode Array Detector		
Wavelength	Ibuprofen: 264 nm/ 4 nm, Ref.: 360 nm/ 100 nm Thalidomide: 254 nm/ 4 nm, Ref.: 360 nm/ 100 nm and 295 nm/ 4 nm, Ref.: 360 nm/ 100 nm	
Data rate	20 Hz	
Cartridge cell	10-mm Max-Light Cartridge Cell	
Second dimension detector, Agilent 1290 Infinity Diode Array Detector		
Wavelength	Ibuprofen: 264 nm/ 4 nm, Ref.: 360 nm/ 100 nm Thalidomide: 254 nm/ 4 nm, Ref.: 360 nm/ 100 nm and 295 nm/ 4 nm, Ref.: 360 nm/ 100 nm	
Data rate	20 Hz	
Cartridge cell	60-mm Max-Light Cartridge Cell	

Valve

The 2-Position/4-Port-Duo valve was equipped with one 60-µL loop and a short cut capillary as shown in Figure 1. The valve was switched automatically when the desired part of the first dimension effluent had to be cut and transferred to the second dimension column (Position 1 in Figure 1). After a defined loop fill time, the valve was switched back (Position 2 in Figure 1), allowing analysis of the content of the loop on the second dimension column. The loop was used in a cocurrent manner (filled and eluted from the same side).

Results and Discussion

The analysis of impurities contained in pharmaceutical substances can be accomplished by subjecting a concentrated solution of the substance to liquid chromatographic analysis. Impurities separated from the pharmaceutical substance are detected as small peaks beside a large peak originating from the main compound.

Ibuprofen

Racemic ibuprofen was chosen to prove the principle of the analysis of impurities in chiral pharmaceutical substances with simultaneous determination of the enantiomeric composition of the API. Figure 2 shows the chromatogram resulting from the first dimension reversed phase analysis of ibuprofen. Here, several impurities (impurities 1–9) are separated from the main compound.

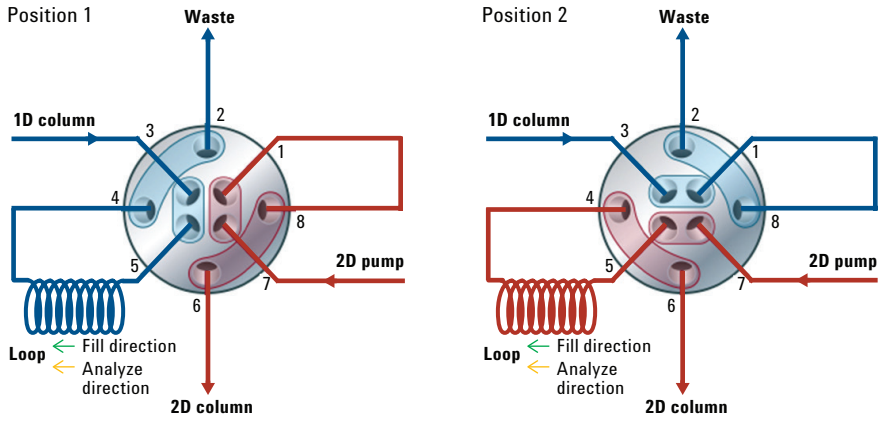

Figure 1. Plumbing diagram of the 2-Position/4-Port-Duo valve.

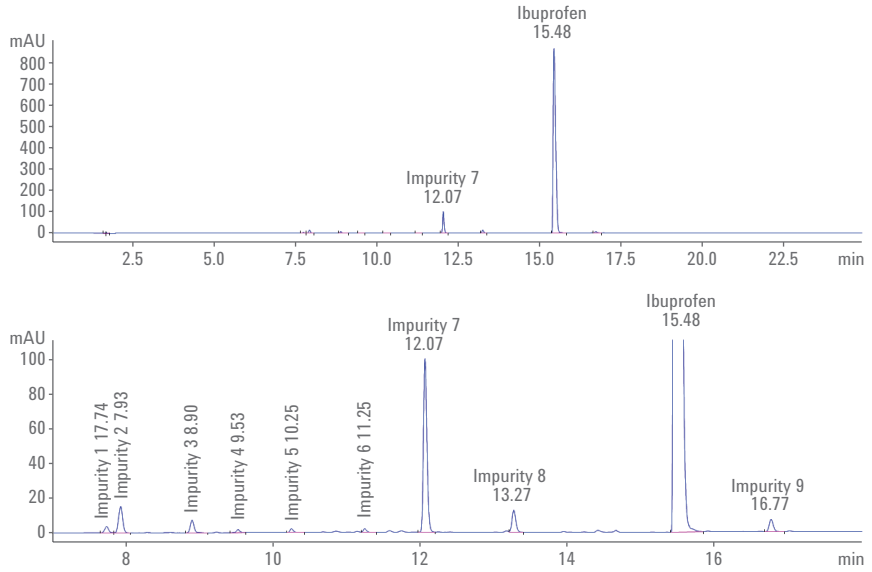

Figure 2. Ibuprofen and impurities 1–9 detected after the first dimension reversed phase separation of a 1 mg/mL solution of ibuprofen sodium salt.

The effluent from the first dimension column was sampled at 15.45 minutes with a loop fill time of 0.20 minutes to transfer the ibuprofen peak to the chiral second dimension column and enable separation of the enantiomers. Figure 3 shows when the effluent of the first dimension column was cut and transferred to the second dimension column (A) and the separation of the ibuprofen enantiomers on the chiral second dimension column (B). The ibuprofen enantiomers were separated with a resolution of Rs = 1.25 on the chiral second dimension column.

To demonstrate the reliability of the heart-cutting process, 10 replicates of the analysis of ibuprofen (1 mg/mL ibuprofen sodium salt) were performed and statistically evaluated. Table 1 shows the good reproducibility of ibuprofen peak retention time and area obtained in the first dimension, and also in the second dimension after the heart-cutting process.

Additionally, the linearity of the amounts of ibuprofen transferred in the heart-cutting process was evaluated in the concentration range of 0.1–1 mg/mL ibuprofen sodium salt. The resulting linearity coefficients (Table 2) show that, reproducibly, the same part of the first dimension ibuprofen peak is transferred to the second dimension column by the heart-cutting process.

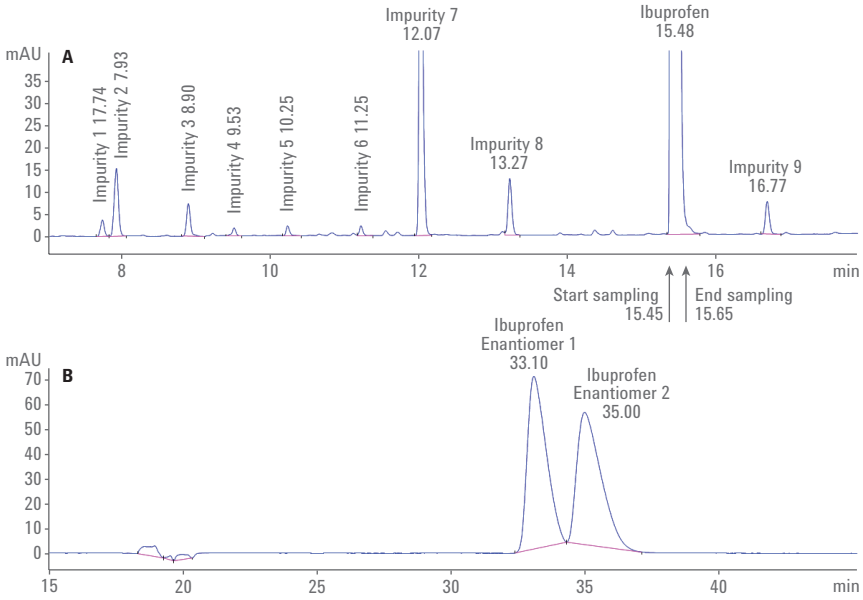

Figure 3. Separation of ibuprofen and impurities 1–9 on the first dimension reversed phase column (A) and heart-cutting of the ibuprofen peak and transfer to the chiral second dimension column for separation of the enantiomers (B) (1 mg/mL ibuprofen sodium salt).

Table 1. Statistical evaluation of the analysis of 1 mg/mL ibuprofen sodium salt (n = 10).

		Second dimension	
	First dimension	Enantiomer 1	Enantiomer 2
Retention time ± S.D.	15.48 ± 0.01 min	33.10 ± 0.07 min	35.00 ± 0.07 min
RSD	0.07 %	0.2 %	0.2 %
Area ± S.D.	4,697 ± 29	3,360 ± 17	3,290 ± 17
RSD	0.6 %	0.5 %	0.5 %

Table 2. Linearity of the analysis of 0.1–1 mg/mL ibuprofen sodium salt after heart-cutting (n = 3 for each concentration).

	R^2
Enantiomer 1	0.9999
Enantiomer 2	0.9999
Sum of enantiomers	0.9999

Thalidomide

In addition to the analysis of ibuprofen as proof of principle, thalidomide was analyzed as an example of a chiral pharmaceutical substance with different pharmacological activity between the enantiomers: R-(+)-thalidomide is a sedative, and S-(–)-thalidomide is a teratogen[7].

Figure 4 shows the analysis of a mixture containing R-(+)-thalidomide as the main compound and S-(–)-thalidomide as the trace compound. To transfer the thalidomide peak from the first dimension reversed phase column to the second dimension chiral column, the effluent from the first dimension column was sampled at 8.20 minutes with a loop fill time of 0.20 minutes. On the second dimension chiral column, the thalidomide enantiomers were separated with a resolution of Rs = 8.3.

The enantiomeric composition of a chiral substance can be described by the enantiomeric excess (ee). It indicates to what extent a chiral substance contains one enantiomer in greater amounts than the other. This means that a racemic mixture has an ee of 0 %, and a pure enantiomer has an ee of 100 %[8].

Based on the peak areas of R-(+)-thalidomide and S-(–)-thalidomide, an enantiomeric excess of 98.1 % of R-(+)-thalidomide can be calculated from the analysis of the mixture containing R-(+)-thalidomide as the main compound and S-(–)-thalidomide as the trace compound. This is in agreement with the theoretical enantiomeric excess that can be calculated from the preparation of the mixture (theoretical ee of 98.1 % of R-(+)-thalidomide in the mixture; the analysis of the R-(+)- and S-(–)-thalidomide standards showed that the standards were not optically pure; R-(+)-thalidomide showed an ee of 99.6 % and S-(–)-thalidomide showed an ee of 93.2 %).

In the thalidomide standards purchased, no impurities could be detected (Figure 4A).

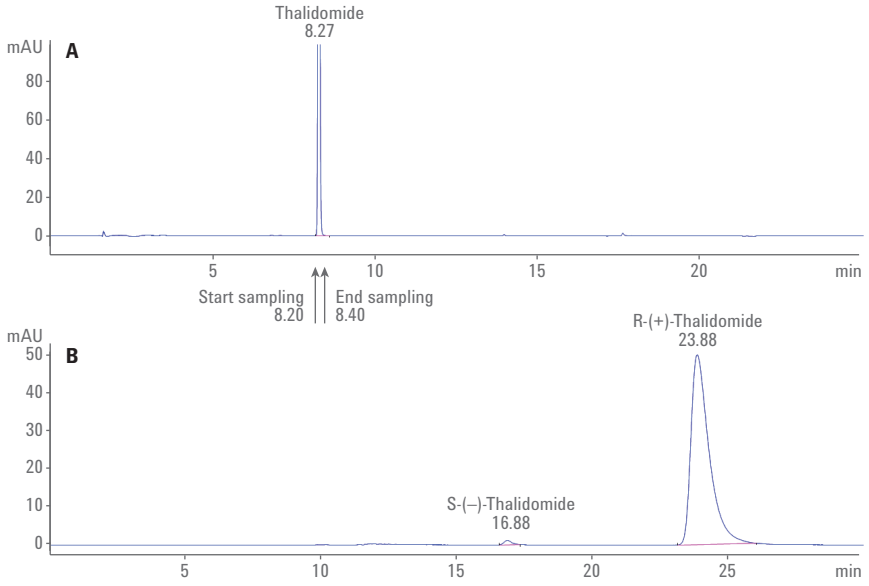

Figure 4. Analysis of a mixture of R-(+)- and S-(–)-thalidomide on the first dimension reversed phase column (A) and heart-cutting of the thalidomide peak and transfer to the chiral second dimension column for separation of the enantiomers (B); detection at 295 nm.

To show the separation of impurities from the main compound thalidomide in the first dimension reversed phase separation, a solution of RS-(±)-thalidomide was degraded under alkaline conditions at 60 °C. The mixture containing R-(+)-thalidomide as the main compound and S-(–)-thalidomide as the trace compound was spiked with the solution of degraded RS-(±)-thalidomide. Figure 5 shows the separation of the degradation products from the main compound thalidomide in the first dimension reversed phase separation.

Conclusion

This Application Note demonstrates that the Agilent 1290 Infinity 2D-LC Solution is ideally suited for the analysis of impurities in chiral pharmaceutical substances and the simultaneous determination of the enantiomeric composition of the API. In the first dimension, a reversed phase separation was used to separate achiral impurities from the API. The API was transferred to a chiral second dimension column for determination of the enantiomeric composition in a heart-cutting experiment. As proof of principle, racemic ibuprofen was analyzed. The reliability of the heart-cutting process is shown by the excellent reproducibility of retention time and peak area obtained from multiple injections of ibuprofen as well as from the good linearity coefficients resulting from the analysis of different concentrations of ibuprofen. Another example shows the simultaneous analysis for thalidomide and the determination of the enantiomeric excess for a mixture containing R-(+)-thalidomide as the main compound and S-(–)-thalidomide as the trace compound.

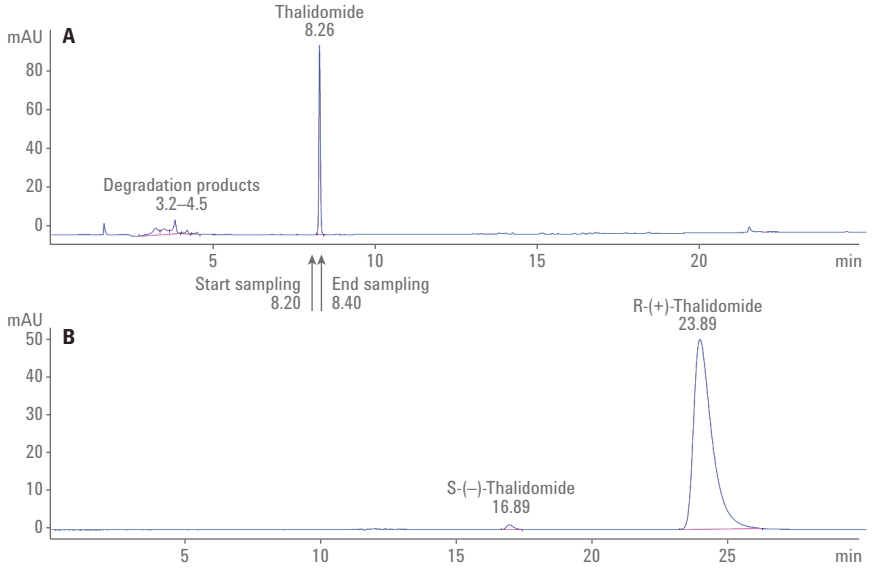

Figure 5. Analysis of a mixture of R-(+)- and S-(–)-thalidomide spiked with thalidomide degradation products on the first dimension reversed phase column (A; detection at 254 nm) and heart-cutting of the thalidomide peak and transfer to the chiral second dimension column for separation of the enantiomers (B; detection at 295 nm).

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