Isolation and purification of enantiomers on the Agilent 1100 Series purification system

Application

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Abstract

In this Application Note the isolation of two enantiomers from a racemic sample of a respiratory drug candidate developed by Boehringer Ingelheim in Biberach, Germany is demonstrated using an Agilent 1100 Series purification system. The initial analytical run, the overloading experiment, the optimization of the preparative scale method and the analysis of the combined collected fractions are also shown.

Introduction

The stereoisomeric composition of drugs has become a key issue in today's drug development. Drug registration requires a pure drug enantiomer during the candidate selection phase to evaluate differences in efficacy, toxicity or pharmacokinetics of the two enantiomers. It is essential to obtain a pure drug enantiomer as early as possible. Often a stereoselective route of synthesis is not available to support early drug discovery activities. Due to the fact that only small amounts of potent drugs are required to carry out the corresponding experiments in early development, preparative high-performance liquid chromatography is a suitable tool to isolate the pure enantiomers from a racemic mixture. Isolation using chromatography as an alternative to the development of an enatioselective synthesis has the benefit that a large number of drug candidates can be made available timely and cost-effectively. In many cases the development of a stereoselective synthesis is very time-consuming. Taking into consideration that the failure rate is very high at the beginning of the drug development process, excessive development costs and especially unnecessary development time can be avoided using preparative high-performance chromatography. In this Application Note the separation of the racemate of a respiratory drug candidate in gram scale is described. The separation is carried out using the Agilent 1100 purification system[1] in combination with a preparative polysaccharide column from Daicel®.

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Chromatographic conditions:

Column:	Chiralpak AD-H 4.6 x 150 mm , 5 µm
Mobile phase:	A: hexane
	B: isopropanol
Isocratic:	at 40 % B
Flow rate:	1 mL/min
Stop time:	30 min
Inj. vol.:	1 µL
Column temp.:	20 °C
DAD:	245 nm /4 (ref. 550/100)
	Flow cell (10-mm path length)
Sample:	1 mg/mL in ethanol

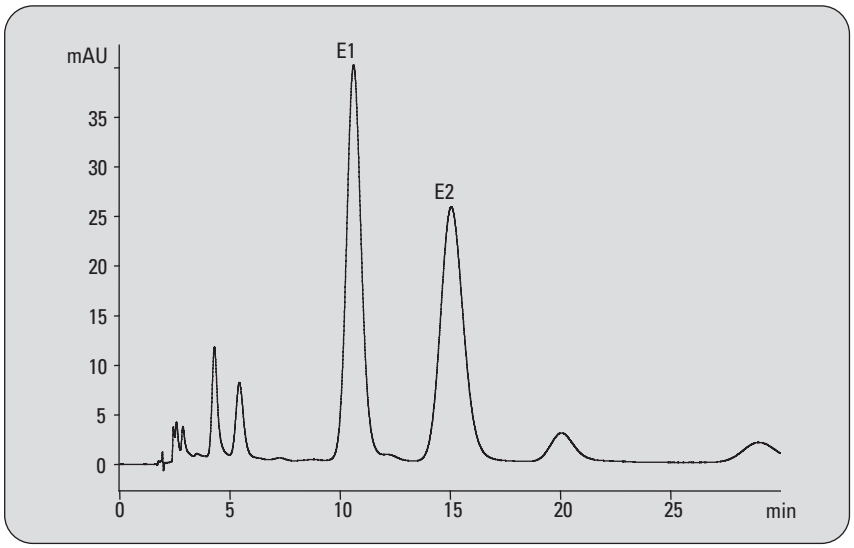

Figure 1
Separation of enantiomers on the analytical column.

Equipment

The experiments were performed on an Agilent 1100 Series purification system containing the following modules:

- Two Agilent 1100 Series preparative pumps
- Agilent 1100 Series autosampler PS
- Agilent 1100 Series diode-array detector (flow cell: 3 mm path length)
- Agilent 1100 Series fraction collector PS
- Agilent 1100 Series 12-position/13-port valve

The system was controlled using the Agilent Chemstation (rev. A.10.01). The 12-position/13-port valve was used for time-based fraction collection[2] after the time-based fraction collection parameters had been validated.

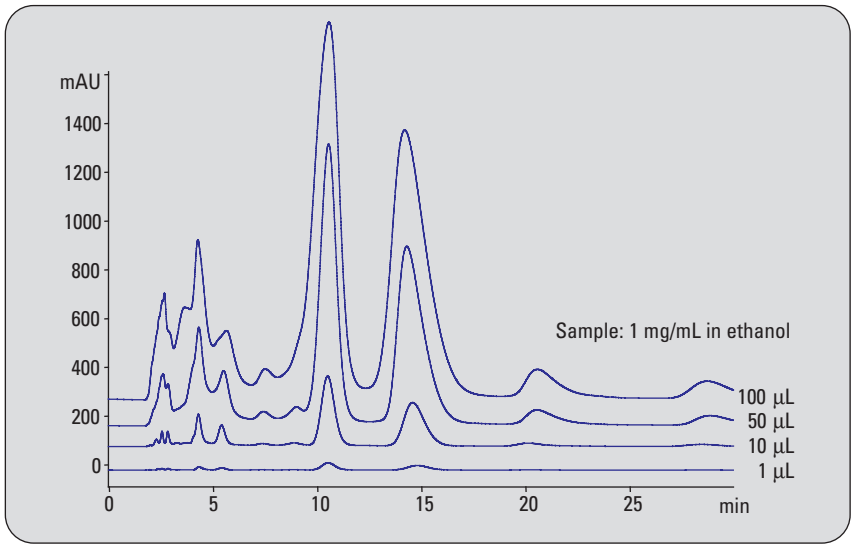

Figure 2
Volume overloading experiments.

Results and Discussion

Analytical method

The analytical method to separate the enantiomers E1 and E2 was developed on a Chiralpak AD-H 5 µm column (4.6 x 150 mm) as shown in figure 1. This method was also used later in the purification process to control the purity and enantiomeric excess of the fractions. The racemic mixture was always injected prior to a batch of fractions to make sure the retention time for the two enantiomers was stable. Experiments were carried out to determine the maximum amount of sample, which can be loaded onto the analytical column. The results of the loading experiments are shown in figure 2. The maximum amount that could be loaded was 0.1 mg on the 4.6 x 150 mm column.

Chromatographic conditions:

Column:	Chiralpak AD 50 x 500 mm, 20 µm
Mobile phase:	A: hexane
	B: isopropanol
Isocratic:	at 40 % B
Flow rate:	40 mL/min
Stop time:	150 min
Inj. vol.:	900 µL
Column temp.:	ambient
DAD:	254 nm /4 (ref. 550/100)
	Flow cell (3-mm path length)
Sample:	20 mg/mL in ethanol

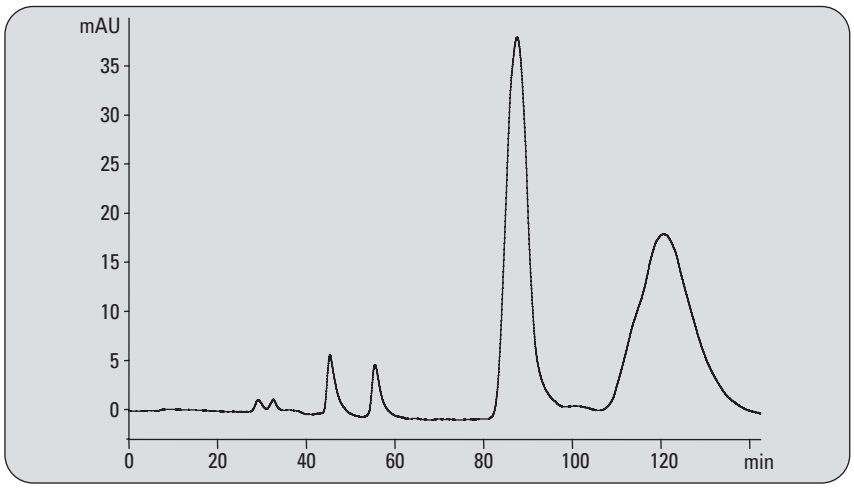

Figure 3
Scale-up to a preparative column.

Chromatographic conditions:

Column:	Chiralpak AD 50 x 500 mm, 20 µm
Mobile phase:	A: hexane
	B: isopropanol
Isocratic:	at 60 % B
Flow rate:	40 mL/min
Stop time:	100 min
Inj. vol.:	900 µL
Column temp.:	ambient
DAD:	254 nm /4 (ref. 550/100)
	Flow cell (3-mm path length)
Sample:	50 and 200 mg/mL in ethanol

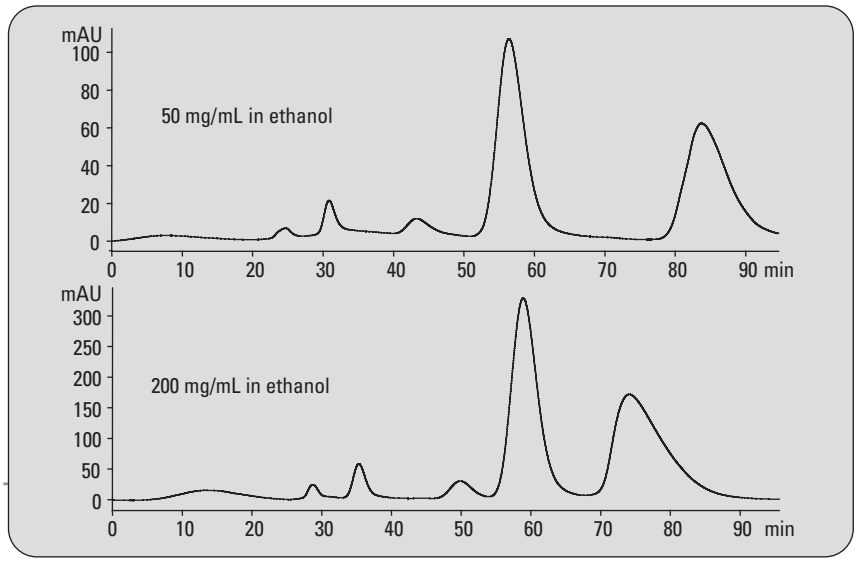

Figure 4
Increasing the sample amount loaded onto the column.

Preparative method

Based on the results of the overloading experiments on the analytical column the scale-up to the preparative column was done. The result of the first run is shown in figure 3. It is not uncommon that direct scale-up calculations are not possible in chiral preparative HPLC. Usually the high flow rate calculated for the large i.d. column cannot be achieved because of the restriction of the maximum backpressure on that column. In addition, as shown in this example, the particle size and the column length are often different from the analytical column. In the example the composition of the mobile phase was changed to 60 % isopropanol to shorten the run time for the preparative run. To increase the throughput the sample concentration was increased to 50 and to 200 mg/mL in ethanol, keeping the injection volume at 900 µL. The results of the runs are shown in figure 4.

Analysis of the combined collected fractions

The combined collected fractions were analyzed using the method as described in *Analytical method*. Both enantiomers could be isolated with very high enantiomeric excess as shown in figure 5.

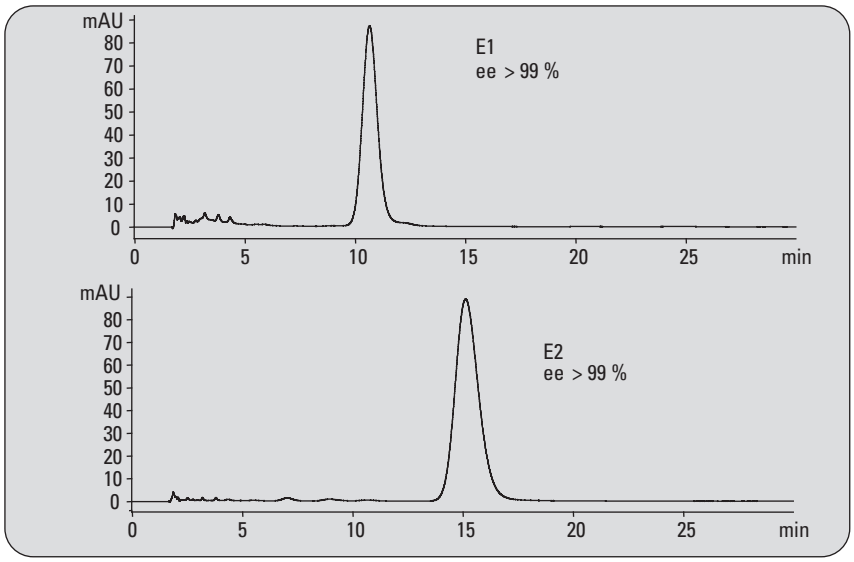

Figure 5
Re-analysis of the combined fractions.

Conclusion

In this Application Note the purification of two enantiomers of a respiratory drug candidate from Boehringer Ingelheim in Biberach, Germany was demonstrated using an Agilent 1100 Series purification system. The method was developed on an analytical column and scaled up to a preparative column after the loading experiments had been done. After adjusting the preparative method the purification was done in gram scale and the combined collected fractions were re-analyzed to determine the enantiomeric excess.

References

1.
"Agilent 1100 Series purification system", Agilent Technologies Brochure, publication number 5989-1255EN **2004**.

2.
"Recovery collection and time-based fraction collection - preparative HPLC with the Agilent 1100 Series valve solutions", *Agilent Technologies Application Note, publication number 5988-8225EN*, **2003**.

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