Determination of Main and Trace Enantiomers in One Run Using the Agilent 1200 Infinity Series HDR-DAD Impurity Analyzer System

Abstract

Chiral compounds are present in many pharmaceutical products, and the determination of each enantiomer during drug development and production is mandatory. The Agilent 1200 Infinity Series High Definition Range Diode Array Detector Impurity Analyzer is the ideal solution to determine trace and main enantiomers with HPLC in one run. Typically, the linear range of the HDR-DAD is extended up to 6,000 mAU. Conversely, it is very sensitive and detects peak heights below 1 mAU with high certainty. This Application Note shows that S-propranolol, with a purity of 99.93 %, was observed in the presence of 0.07 % R-propranolol. Only one run was needed to quantitate both compounds and determine the enantiomeric excess (ee) value.
Introduction

The determination of enantiomers in pharmaceutical products is mandatory to avoid dangerous side effects due to differing modes of action between two enantiomers.

The following are official recommendations given by the US Food and Drug Administration:

The stereoisomeric composition of a drug with a chiral center should be known and the quantitative isomeric composition of the material used in pharmacologic, toxicologic, and clinical studies should be known. Specifications for the final product should assure identity; strength, quality, and purity from a stereochemical viewpoint.

To measure the purity of chiral compounds, the enantiomeric excess (ee) is calculated. It reflects the degree to which a sample contains one enantiomer in greater amounts than the other. A racemic mixture of equal amounts has an ee of 0 %, while a single pure enantiomer has an ee of 100 %. A sample with 70 % of one enantiomer and 30 % of the other has an ee of 40 %.

In the following application, we determine a main enantiomer with an ee < 99.9 % and the related trace enantiomer in one run using the Agilent 1200 Infinity Series HDR-DAD Impurity Analyzer System.

The HDR-DAD provides a wide linear range and at the same time high sensitivity. This allows the determination of main compounds up to 6,000 mAU and impurities below < 1 mAU in one run without the need to adapt the injection volume for the different concentrations. Only one injection is needed to detect and quantify low and high concentrated compounds.

As an example, R (+)-propranolol (trace compound) and S (–)-propranolol (main compound) was chosen. The (–) isomer is 60–100 times more active than the (+) isomer in blocking the inotropic, chronotropic and vasodepressor actions of isoprenaline. The (–) isomer is much more active at blocking β-adrenergic stimulation.

Experimental

The following instruments were used:

- Agilent 1200 Infinity Series HDR-DAD Impurity Analyzer System consisting of:
  - Agilent 1290 Infinity DAD with 60-mm cell (G4212A)
  - Agilent 1290 Infinity DAD with 3.7-mm cell (G4212A)
  - Agilent 1200 Infinity HDR-DAD Solution Kit (G2199AA)
  - Agilent 1290 Infinity Thermostatted Column Compartment (G1316C)
  - Agilent 1290 Infinity Autosampler (G4226A)
  - Agilent 1290 Infinity Thermostat (G1330B)
  - Agilent 1290 Infinity Binary Pump (G4220A)

Chromatographic conditions

<table>
<thead>
<tr>
<th>Column</th>
<th>250 × 4.6 mm, YMC Chiral Cellulose-C, 5 µm (provided by customer)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow rate</td>
<td>1 mL/min</td>
</tr>
<tr>
<td>Mobile phases</td>
<td>n-hexane/2-propanol/DEA* (80/20/0.1) isocratic out of one bottle</td>
</tr>
<tr>
<td>Temperature</td>
<td>25 °C</td>
</tr>
<tr>
<td>Detection</td>
<td>230/10 nm, ref 400/100 nm, 10 Hz</td>
</tr>
<tr>
<td>Injection volume</td>
<td>5 µL, 10 °C sample temperature</td>
</tr>
</tbody>
</table>

*DEA = diethylamine

Concentrations for testing linearity and LOD of S (–)-propranolol

<table>
<thead>
<tr>
<th>Calibration level</th>
<th>Amount (ng/5 µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>3.424 (LOD)</td>
</tr>
<tr>
<td>1</td>
<td>17.12</td>
</tr>
<tr>
<td>2</td>
<td>86.5</td>
</tr>
<tr>
<td>3</td>
<td>428</td>
</tr>
<tr>
<td>4</td>
<td>2,140</td>
</tr>
<tr>
<td>5</td>
<td>10,700</td>
</tr>
</tbody>
</table>

Sample preparation

1. 10.7 mg of S-propranolol was dissolved in 1 mL of ethanol, then diluted 1:5 with ethanol, and 10.7 µg/5 µL were injected.
2. 1.5 mg R-propranolol was dissolved in 1 mL of ethanol, then diluted 1:4,000 with ethanol, and 1.875 ng/5 µL were injected to determine the LOD.
3. The mixture to determine the ee value contained 10,700 ng of S-enantiomer and 7.5 ng of R-enantiomer.
4. ee = 99.87 % for S (–)-propranolol

Analyzed compound

R (+)-propranolol (trace compound) and S (–)-propranolol (main compound) purchased from Sigma-Aldrich, Germany.
**Results and Discussion**

The HDR-DAD system is configured during instrument configuration. Both detectors are clustered and, in the user interface, appear as one detector, see Figure 1.

To evaluate the method performance and determine the ee value for the main enantiomer, the following experiments were applied:

- Evaluation of linearity, LOD, and precision of the applied method
- Analysis of enantiomeric mixture using the 1290 Infinity Binary LC with HDR-DAD. The S-enantiomer had a purity of $99.93\%$, and the R-enantiomer was present in the mixture as a trace compound of $0.07\%$.

The precision of the method was determined over five runs using the racemate with equal concentrations. The precision of retention times (RT RSD) was $\sim 0.1\%$ over five consecutive runs, and the area precision was $0.25$ and $0.64\%$ over five consecutive runs, see Figure 2.

The LOD was evaluated for both enantiomers, see Figure 3. The LOD was as low as $2.34$ ng for the R-enantiomer and $3.64$ ng for the S-enantiomer.

---

**Figure 1.** User interface in OpenLAB CDS.

**Figure 2.** Precision of retention times and area for propranolol enantiomers, overlay of five runs.

**Figure 3.** LOD of propranolol enantiomers.
Linearity was tested for the S-enantiomer from 17.12 to 10,700 ng, see Figure 4. The correlation was 0.99996.

Determination of the ee value in one run for main and trace enantiomers was possible due to the wide linear range of the HDR-DAD, even though both enantiomers had significantly different concentrations, and respectively different peak heights, see Figure 5. Typically, peaks as high as ~3,700 mAU, as those obtained for S-propranolol, exceed the linear range of a conventional UV detector. Another benefit of the HDR-DAD is that trace compounds are easier to detect and quantified if peak heights are increased by higher injection volumes. After injecting 5 µL of the enantiomer mixture, the R-enantiomer was reliably quantified with a peak height of ~14 mAU.

The ee value was evaluated to 99.87 % for the S (–)-propranolol.

**Conclusion**

The Agilent 1200 Infinity Series HDR-DAD Impurity Analyzer System enabled the analysis of chiral compounds with one main enantiomer and one trace enantiomer in one run. The wide linear range of the HDR-DAD allowed the reliable quantitation of the main peak with ~3,700 mAU, and of the trace compound with ~14 mAU. Consequently, the ee value was evaluated without requiring two injections with different volumes. With a conventional UV detector, the main enantiomer requires a low injection volume and the trace enantiomer requires a high injection volume to be able to quantify both compounds with high certainty.

**References**


