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Fast UHPLC separation of budesonide diastereomers

Authors

Katherine Lovejoy, Mauro De Pra, and Frank Steiner
Thermo Fisher Scientific, Germering, Germany

Keywords

Active Pharmaceutical Ingredient (API), USP, corticosteroid, Vanquish Flex, Accucore XL

Goal

Development of a fast assay for budesonide diastereomers based on isocratic UHPLC with a solid core reversed phase column

Introduction

Budesonide is a synthetic corticosteroid that is available as mixture of two diastereomers, 22R and 22S. The 22R form is two times as active as the 22S, and the ratio of the two diastereomers in medicinal products is therefore controlled by regulatory agencies. In contrast to enantiomers, which are chemically identical, diastereomers are chemically different and can be separated in achiral systems. Separation of the budesonide isomers is nonetheless difficult due to the very similar interaction of the almost identical molecules with the stationary phase. Because of this similar retention behavior, assays for active pharmaceutical ingredients (API) containing isomeric impurities are normally developed as isocratic methods rather than gradient methods. The USP monograph for the budesonide assay is an isocratic method that requires the fulfillment of three parameters: the plate count for R-budesonide must be at least 5500, the resolution between the two peaks must be at least 1.5 and the retention time of S-budesonide must be 1.1 times that of R-budesonide.[1]

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In this work, an assay for budesonide was developed using a Thermo Scientific™ Accucore™ XL C18, 4 µm column, operated with a Thermo Scientific™ Vanquish™ Flex Quaternary UHPLC system. The solid core technology of the Accucore XL C18 column allowed fast and efficient separation of budesonide diastereomers. The Vanquish Flex Quaternary system provided the flexibility and reliability required to develop and optimize methods for the budesonide API analysis. This work describes the fine tuning of the challenging isocratic separation based on kinetic and thermodynamic analysis.

Experimental

Instrumentation

- Vanquish Flex Quaternary system:
  - Quaternary Pump, Vanquish Flex, P/N VF-P20-A, with 150 µL mixer
  - Split Sampler FT, P/N VF-A10-A
  - Column Compartment, P/N VH-C10-A
  - UV Detector, VWD F, P/N VF-D40-A, with 2.5 µL flow cell, 7 mm, P/N 6077.0360
- Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) software version 7.2

Chemicals and consumables

- Acetonitrile, Optima™ LC-MS grade, Fisher Chemical (P/N A955-212)
- Deionized water, Thermo Scientific™ Barnstead™ GenPure™ xCAD Plus Ultrapure Water Purification System (P/N 50136149)
- Accucore XL C18, 4 µm, 3 × 150 mm (P/N 74104-153030)
- Budesonide, >99%, Sigma®, CAS 51333-22-3 (P/N B7777)

Separation conditions

The separation conditions are listed in Tables 1 and 2.

Table 1. Chromatographic conditions used in all experiments.

Column	Accucore XL C18, 4 µm, 3 × 150 mm
Mobile phase	60% water (18.2 MΩ at 25 °C), 40% acetonitrile
Injection volume	1 µL
Detection	244 nm, 2.5 µL flow cell, data collection rate 20 Hz, response time 0.20 s

Table 2. Optimized chromatographic conditions.

Column	Accucore XL C18, 4 µm, 3 x 150 mm
Mobile phase	60% water, 40% acetonitrile, pre-mixed in channel A
Flow rate	0.64 mL/min
Temperature	30 °C, forced air, Active pre-heater: 30 °C
Injection volume	1 µL
Detection	Variable wavelength detector 244 nm, data collection rate 20 Hz, response time 0.20 s 2.5 µL flow cell
Analytes	1) R-Budesonide, Diastereomer B 2) S-Budesonide, Diastereomer A
Run time	2.5 minutes

Results and discussion

Initial solvent screening showed that 40% acetonitrile in water provided values for resolution, plate number (N), and relative retention time (RRT) that fulfilled the compendial requirements (Figure 1). The initial flow rate was 0.52 mL/min and the run time was 5 minutes with separation of the diastereomers at 3 minutes.

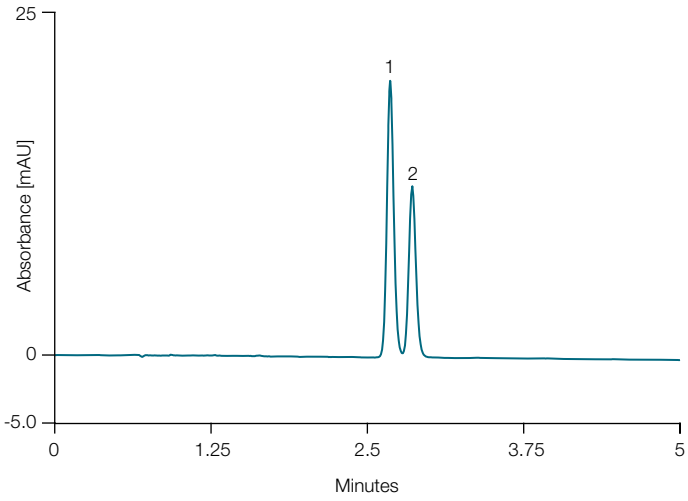


Figure 1. Initial method for the assay of budesonide. Flow rate 0.52 mL/min. Temperature 30 °C. Other conditions as in Table 1.

Flow rate optimization

Even though the method fulfilled the requirements and was sufficiently fast, further method optimization possibilities were explored. The first optimization step was to evaluate the dependence between flow rate and efficiency of the budesonide peak. Figure 2 shows the Van Deemter plot measured at 30 °C (blue trace). Observation of the plot indicates that the flow rate chosen to run the mobile phase optimization, 0.52 mL/min, is greater than the flow rate at the minimum of the Van Deemter curve. The flow rate for optimal efficiency is 0.106 mL/min. The separation of the budesonide diastereomers at a flow rate close to optimum would produce very efficient peaks, with more than 19,000 plates and resolution of 1.95. However, the run would take 14 minutes to separate the diastereomers at this decreased flow rate (Figure 3a). Because the minimum requirements of efficiency and resolution are easily met with a fast run, working close to the optimal flow rate is not recommended for this specific assay.

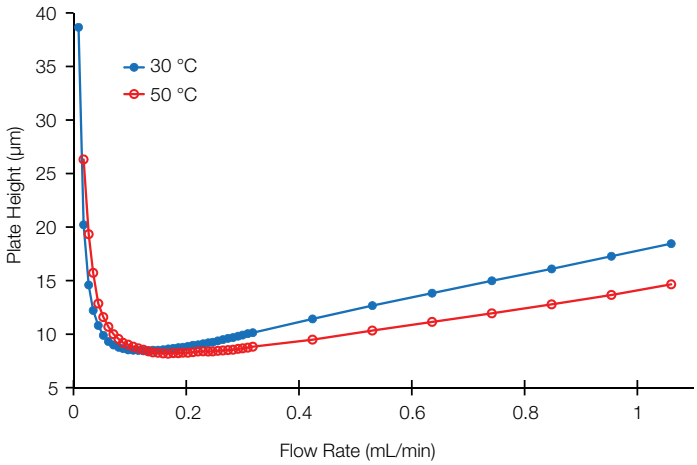
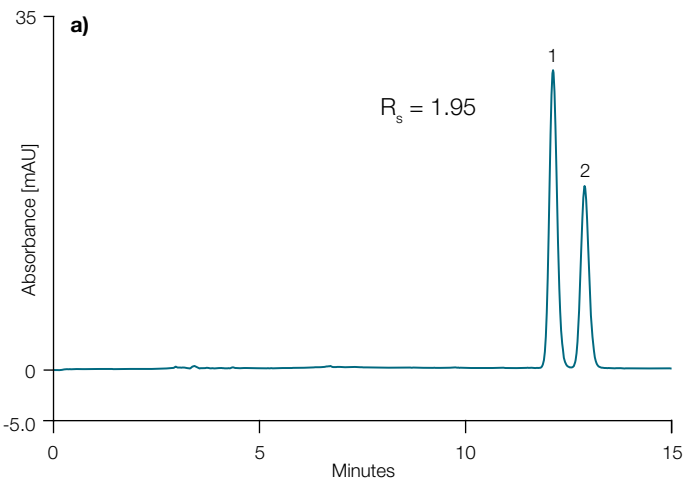
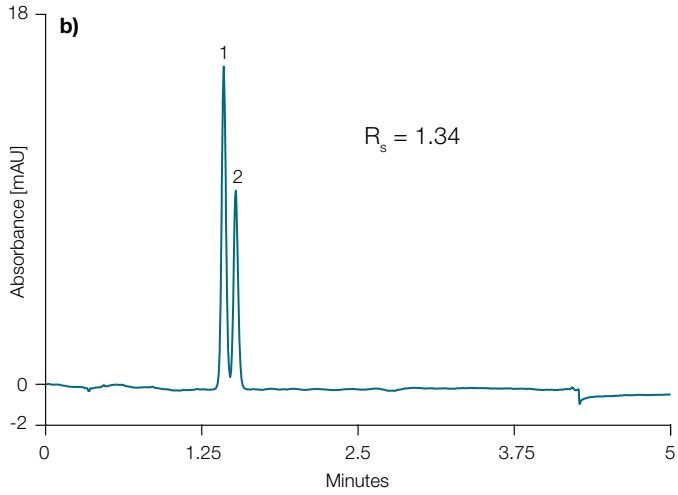


Figure 2. Van Deemter plot for the budesonide diastereomers. The plot shows plate height of the R-budesonide peak at different flow rates, at 30 °C (blue trace) and at 50 °C (red trace). Conditions listed in Table 1.





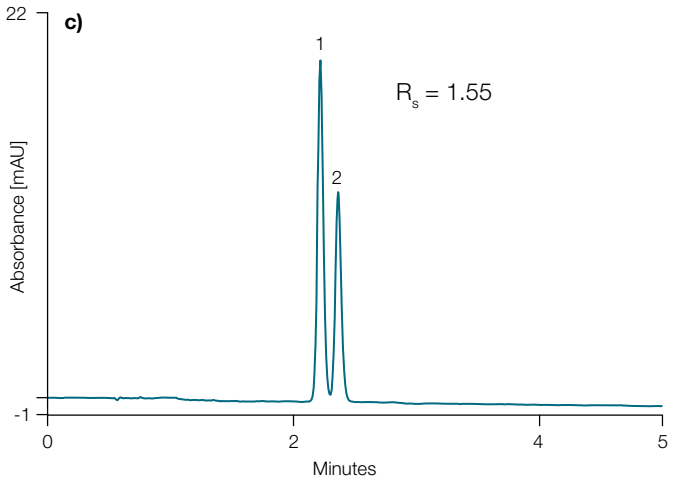


Figure 3. Chromatograms of the R- and S-budesonide separation at 30 °C and at different flow rates. (a) A flow rate of 0.106 mL/min provided the best possible resolution. (b) 1.06 mL/min was the highest flow rate examined, but results in unacceptably low resolution. (c) 0.64 mL/min was identified as the highest possible flow rate that still provided acceptable resolution. Conditions listed in Table 1.

In fact, because of the relatively flat behavior of the Van Deemter plot at high flow rate, caused by the small C term associated with the solid core particle column, the method run time can be decreased further. Equation 1 describes the relationship between plate height (H), plate number (N), and column length (L). The separation at 0.52 mL/min delivered more than 10,000 plates, which corresponds to H = 15 µm. This plate count of 10,000 is 1.8 times larger than the USP requirement of 5500 plates (Table 3). Applying this information, the Van Deemter equation can be used to estimate the fastest flow rate to fulfill the efficiency requirement of H = 27 µm. Although the Van Deemter curve does not extend this far, we know the flow rate would be above 1 mL/min, and the method at this flow rate would separate the diastereomers in less than 1.5 minutes (Figure 3b). However, resolution also changes with flow rate. The relationship between resolution (Rs), selectivity (α), and retention factor (k') is described in Equation 2.

$$\text{Equation 1: } H = \frac{L}{N}$$

$$\text{Equation 2: } Rs = \frac{\sqrt{N}}{4}\,\frac{(\alpha-1)}{\alpha}\,\frac{k'}{(k'+1)}$$

$$\text{Equation 3: } RRT = \frac{RT_2}{RT_1} = \frac{1+k_2'}{1+k_1'}$$

Of all the terms in Equation 1, only N depends on the flow rate. The Van Deemter equation can be used to predict the change of resolution with flow rate by easily combining Equation 1 and Equation 2. The flow rate of 0.64 mL/min is the fastest separation that could be achieved to fulfill the compendium requirements of both resolution and efficiency. This flow rate is within the USP's adjustment limits of +50% for isocratic methods, according to the <621> guidance. For this flow rate, the total analysis time, without the injection cycle, is 2.5 minutes.

Temperature optimization

Theory predicts that the method could be sped up by raising the temperature, which should improve efficiency at higher flow rates. The method, when run at a higher temperature and higher flow rate, would then be faster than and at least equally as efficient as the slower method. Figure 1 shows the Van Deemter measured at 50 °C (red empty circles). The comparison between the Van Deemter at 30 and 50 °C clearly points to the advantages of running chromatography at high temperature when fast methods are needed. In fact, the dependency of plate height, and therefore resolution, on flow rate is less steep at 50 °C. With this consideration we could conclude that further method optimization should be made at 50 °C. However, the monograph also dictates a minimum value for the RRT. As mentioned above, the RRT requirements were fulfilled at 30 °C. The RRT is described by Equation 3, where RT_1 is the retention time and k'_1 is the retention factor. Equation 3 shows that as long as the retention factors of the isomers do not change, the RRT remains constant. The influence of flow rate on retention factors is assumed constant in these experiments, therefore we can freely select the flow rate across the Van Deemter curve without significantly affecting the RRT. Temperature, on the other hand, influences the retention factor.

Because the diastereomers are chemically and structurally similar, it could be expected that the temperature dependence of their retention factors would also be similar. The Van't Hoff plot of Figure 4 shows that this is not true. The natural log of retention factor is linearly dependent on 1/T for both diastereomers, which is a common behavior in chromatography. However, the lines are not parallel, and the retention factors become closer as the temperature increases. The practical consequence is that the RRT also decreases with temperature. The minimum RRT of 1.1 is fulfilled only for temperature of 30 °C or lower for the mobile phase composition used in these experiments. In this case, the method cannot be accelerated further by increasing temperature, and the initial temperature of 30 °C is selected. Optimized conditions are listed in Table 2.

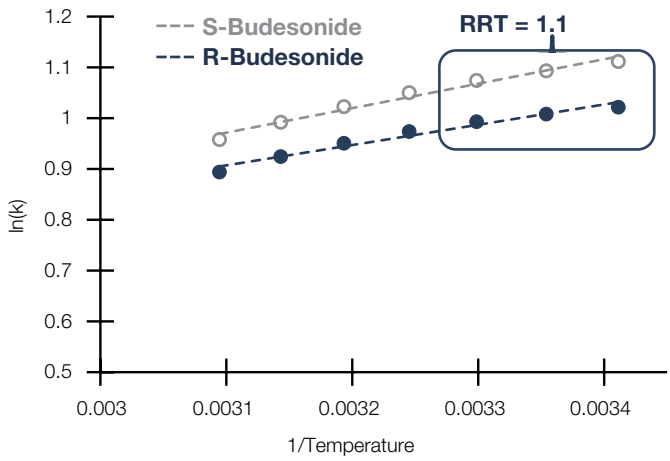


Figure 4. Van't Hoff plot for the budesonide diastereomers.

Table 3. USP requirements for the assay of budesonide drug substance by HPLC and performance of the optimized UHPLC method.

Parameter	USP requirements	Optimized method
Peak resolution	≥ 1.5	1.5
Theoretical Plates R-budesonide	≥ 5500	10,485
RRT	1.1	1.1

Conclusion

In this work, an assay for the quantification of budesonide diastereomers was developed with a Vanquish Flex Quaternary system fitted with an Accucore XL C18 column. It was shown how simple kinetic and thermodynamic tools, namely Van Deemter and Van't Hoff plots, can be used to speed-up a difficult isocratic separation. Although full Van Deemter plots were recorded in this work for educational purposes, such an extensive evaluation of flow rate influence is not needed for method optimization in practice. A few data points at flow rates above the minimum are usually sufficient to assess the separation performance at a high flow rate.

The findings also illustrate that separation speed optimization through elevated column temperature may sometimes fail, namely when selectivity of a critical pair is reduced with increasing temperature.

The selection of the Accucore XL C18 column allowed the use of a flow rate five times greater than the optimum due to the flatter Van Deemter plot associated with the low C-term value characteristic of solid core particles. This increased flow rate greatly increases the potential method throughput even at lower column temperatures. In general, Accucore columns allow excellent separation efficiency with limited back pressures. The final method back pressure was only 120 bar, which is easily attainable with standard HPLC instrumentation.

Reference

1. *United States Pharmacopeia, Revision Bulletin.* "Budesonide." June 1, 2011, 1-2 [Online] http://www.uspnf.com/official-text/accelerated-revision-process/accelerated-revision-history/budesonide (accessed March 20, 2017).

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