INTRODUCTION

The demands on the analytical laboratory to qualitatively and quantitatively determine the impurities in these active pharmaceutical ingredients and degradation products continue to increase. The FDA regulations require companies to develop methods for analysis and characterization of these impurities that could arise from the synthesis process, raw material provider, and degradation products. The utilization of UPLC or PDA data alone for these analyses is often inadequate. Complications arise in many cases, due to the lack of specificity in the UV absorption spectra. The impurities of interest may not have a detectable UV absorbance whether from lack of chromophores or low level UV concentration exhibits poor UV spectral quality and peak purity becomes more difficult to identify when co-elution occurs. Mass spectrometry analysis becomes more essential as an aid for impurity content reporting due to the increase in sensitivity for impurity content reporting from below 0.1% to 0.05% today with expectations to decrease as regulations for impurity content reporting continues to decrease from 0.1% a few years ago to 0.05% today with expectations to decrease as regulations improve. The impurities of interest provide a higher understanding to the relationship of the impurity/parent origin. We will demonstrate the utility of a UPLC–PDA–MS system and show the significant benefits in resolution, speed, and sensitivity using the ACQUITY UPLC™ system and how this configuration will impact the identification of pharmaceutical grade and related substances.

To best illustrate this concept, we will analyze the pharmaceutical active pharmaceutical ingredients (APIs) used in the production of budesonide and related substances for use with UPLC–MS. The method was used to determine various required quality system performance attributes; e.g., resolution, selectivity, purity, and degradation products. The impurity profiles of multiple batches from three manufacturers of pharmaceutical grade budesonide were assessed and tested for EP related impurity compliance. Exact mass MS data will be collected to determine similarities/differences between the impurity profiles of the various budesonide lots. A high abundance mass match with high confidence is possible to predict molecular elemental composition, it is possible to predict molecular elemental composition.

UPLC–MS METHOD COMPATIBILITY

There are three published methods for the separation of budesonide and the related impurities. Two of these methods are not "MS compatible" due to the use of phosphate buffers.(1-3) The European Pharmacopeia requires the following system suitability specifications based on a 500µg/mL budesonide test solution and reference solutions: (a) The resolution between the R-epimer and S-epimer is not less than 1.5 x 10^13; (b) the retention factor for the S-epimer is less than 1.5; (c) the symmetry factor for the R-epimer is less than 1.5; (d) the centroid of the peak area of the two epimers at 0.05% today with expectations to decrease as regulations improve. The impurities of interest provide a higher understanding to the relationship of the impurity/parent origin. We will demonstrate the utility of a UPLC–PDA–MS system and show the significant benefits in resolution, speed, and sensitivity using the ACQUITY UPLC™ system and how this configuration will impact the identification of pharmaceutical grade and related substances.

EXPERIMENTAL

Materials

Budesonide: Waters Corporation, Massachusetts, USA.

Analytical Standards: (A) Budesonide; (B) Budesonide EP Solution; (C) Budesonide EP Standards; (D) RB2362 (research grade). Sigma Chemical Co. (St. Louis, MO); lot numbers: UI0628 (EP Rx grade); 98.0% - 102.0% and lot # 04507AC. Formic acid 98%-100%; Reidel–DeHaven. 

Sigma: (E) Budesonide: Spectrum quality products (New Brunswick, NJ); Spectrum Chemical, lot#RB2362 (St. Louis, MO); batch # 04507AC. Formic acid 98%-100%; Riedel–DeHaven. 

Spectrum: (F) Budesonide: Spectrum quality products (New Brunswick, NJ); Spectrum Chemical, lot#RB2362 (St. Louis, MO); batch # 04507AC. Formic acid 98%-100%; Riedel–DeHaven. 

Spectrum: (G) Budesonide: Spectrum quality products (New Brunswick, NJ); Spectrum Chemical, lot # UI0628 (EP Rx grade); 98.0% - 102.0%. 

Sample Preparation

The EP related substances test as described in the Budesonide EP monograph was performed on four different vendors batches of budesonide which were purchased from four different vendors. Test solutions (500µg/mL) were prepared for each batch lot. Each test solution was diluted to yield two reference solutions each with concentration of 500µg/mL and 5µg/mL respectively. The total impurities were determined for each lot using the 500µg/mL test solution. The requirements of the minimum European Pharmacopeia specifications are met.

UPLC Conditions

Table 1:

<table>
<thead>
<tr>
<th>Name</th>
<th>Retention Time</th>
<th>Resolution</th>
<th>Symmetry Factor</th>
<th>Signal/Noise</th>
<th>EP Plates</th>
</tr>
</thead>
<tbody>
<tr>
<td>R-epimer</td>
<td>5.073</td>
<td>N/A</td>
<td>1.05</td>
<td>10262</td>
<td>17011</td>
</tr>
<tr>
<td>S-epimer</td>
<td>5.476</td>
<td>2.46</td>
<td>1.02</td>
<td>8646</td>
<td>17390</td>
</tr>
</tbody>
</table>

Figure 1: A full scan injection of 500µg/mL budesonide EP grade (Spectrum Quality Products) standard solution. UPLC method conditions: ACQUITY BEH C18 2.1 x 50 mm, 1.7µm; mobile phase: 68% 20mM Ammonium formate, 32% acetonitrile; flow rate: 0.8 mL/min; injection volume: 5 µL. The additional peaks in trace 1 were identified as impurity peaks above 0.05% EP.

CONCLUSIONS

An Ultra Performance LC–MS method for budesonide and the related impurities was developed. European Pharmacopeia specifications based on the use of phosphate buffers for the USP method are not "MS compatible" due to the use of phosphate buffers. The European Pharmacopeia specifies the following system suitability specifications based on a 500µg/mL budesonide test solution and reference solutions: (a) The resolution between the R-epimer and S-epimer is not less than 1.5 x 10^13; (b) the retention factor for the S-epimer is less than 1.5; (c) the symmetry factor for the R-epimer is less than 1.5; (d) the centroid of the peak area of the two epimers at 0.05% today with expectations to decrease as regulations improve. The impurities of interest provide a higher understanding to the relationship of the impurity/parent origin. We will demonstrate the utility of a UPLC–PDA–MS system and show the significant benefits in resolution, speed, and sensitivity using the ACQUITY UPLC™ system and how this configuration will impact the identification of pharmaceutical grade and related substances.