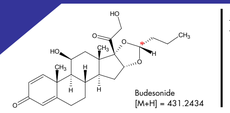
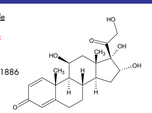
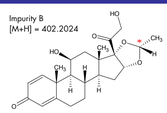
Utility of a UPLC/PDA/MS System for Impurity Profiling Analysis of Pharmaceuticals

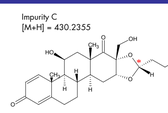
Waters

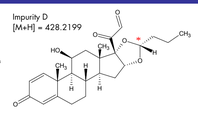


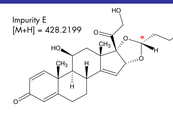
Known Budesonide Impurities
(*) Denotes epimeric

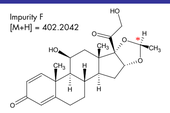


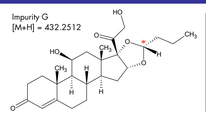












Michael D. Jones and Robert S. Plumb
Waters Corporation, Massachusetts, USA

Introduction

The demands on the analytical laboratory to qualitatively and quantitatively determine active pharmaceutical ingredients and degradants continues to increase. The FDA regulations require companies to develop methods for their analysis and characterization of the APIs, as well as the impurities/degradants that could arise from the synthesis process, raw material provider, and/or storage conditions. The utilization of UV or PDA data alone for these analyses is often inadequate. Complications arise in many situations where compounds have (a) poor to no UV absorbance whether from lack of chromophores or (b) low level impurity concentrations exhibit poor UV spectral quality and peak purity becomes more difficult to identify when co-elution occurs. Mass spectral analysis becomes more essential as FDA regulations for impurity content reporting continues to decrease from 0.1% a few years ago to 0.05% today with expectations to decrease as instrumentation detection limits and techniques continue to become more sensitive. The importance of exact mass and MS fragmentation products to determine the structure of degradants/impurities provides a higher understanding to the relationship of the impurity/degradant 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 pharmaceuticals and their related substances.

To best illustrate this concept, we will analyze the pharmaceutical drug substance; budesonide. Budesonide is a glucocorticosteriod used for the treatment of asthma *via* various matrices and inhalation mechanisms. The official European Pharmacopeial assay was used as a guidance for the redevelopment of the budesonide assay and related substances for use with UPLC—MS. The new method will be used to determine various required qualitative system performance (eg; resolution, S/N, theoretical plates, tailing, symmetry factors). We will also demonstrate the quantification benefits (eg; limits of detection, limits of quantification) of this configuration. The impurity profiles of multiple batches from three manufacturers of pharmaceutical grade budesonide will be 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 vendor batches. This increased performance makes UPLC/PDA/MS the ideal tool for purity profiling.

Experimental

Materials:

Budesonide: Spectrum quality products (New Brunswick, NJ); lot numbers: UI0628 (EP Rx grade); 98.0% - 102.0% and lot # RB2362 (research grade). Sigma Chemical Co. (St. Louis, MO); lot 81K1654; 99%. Molekula; batch# 52459 (Dorset, UK).

Reagents: Acetonitrile Optima; Fisher Scientific (Fairlawn, NJ); Lot#050580. Ammonium formate 97%; Sigma-Aldrich (St. Louis, MO); batch # 04507AC. Formic acid 98%-100%; Reidel—deHaën.

Instrumentation

UPLC Conditions
Instrument: ACQUITY UPLC
Column: ACQUITY UPLC™ BEH C_{18}
Dimensions: 100 x 2.1mm, 1.7µm
Mobile Phase: 68% 20mM Ammonium formate (pH 3.6)/32% acetonitrile
Flow Rate: 0.60 mL/min
Temperature: 40° C
njection Volume: 5 µL; full loop injection mode
Weak Wash: 68:32 (water: acetonitrile) 600 µL
Strong Wash: none
Detection: ACQUITY PDA @ 240 nm with High sensitivity flow cell
Software: Empower™ 2 CDS

MS Conditions
Instrument: Waters LCT-Premier XE™
Software: Masslynx™ 4.1

Tune Page Parameters:
Source: ES+
Capillary (V): 3.2
Sample Cone (V): 35 for reference 20 for analyte
Extraction Cone (V): 4.5
Desolvation Temp (°C): 350.0
Source Temp (°C): 150.0
Cone Flow (L/Hr): 0.0
Desolvation Flow (L/Hr): 800.0

Tof Settings
Acquisition Range: 100 - 800 Da
Scan Time: 0.20 s
Interscan delay: 0.05 s
Lock mass: 100 fmol leucine/enkephalin @ ~20 µL/min

UPLC-MS Method Compatibility

Resulting Method

There are three published methods for the separation of budesonide and the related impurities.[1-3] Two of these methods are not "MS compatible" due to the use of phosphate buffers.[2,3] The European Pharmacopoeia requires the following system suitability specifications based on a 500 µg/mL budesonide test solution and reference solutions: (a) The resolution between the R-epimer and S-epimer is not less than 1.5 (b) run time 1.5x the retention of S-epimer, (c) the symmetry factor for the R-epimer peak is less than 1.5, (d) the theoretical plates calculated for the R-epimer peak is at least 4000, (e) and after six injections of the 500 µg/mL reference solution the %RSD of the sum of the peak areas of the two epimers is at most 1.0%.[2] In converting the method to UPLC we evaluated four currently available hybrid UPLC column chemistries, various pH, buffer concentrations, and temperatures. There was no significant change in separation when the pH was varied from 2.5 - 4.0, neither for pH 9.0 10 mM ammonium bicarbonate nor for buffer concentrations from 10 mM to 25 mM .The resulting method showed the ACQUITY BEH C_{18} 2.1 x 100 mm column provided the best separation of the impurities from the budesonide API at 40°C (Figure 1). 20 mM ammonium formate buffer to pH 3.2 with formic acid was chosen to keep pH consistant with the EP method.

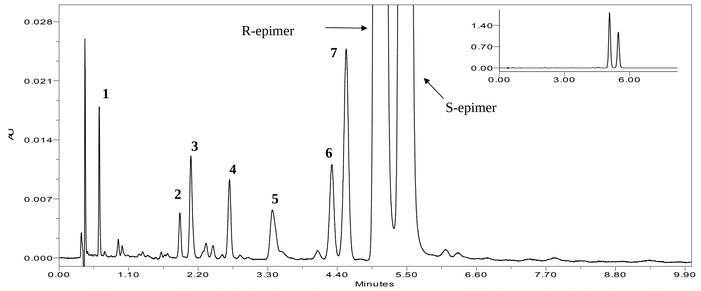


Figure 1: A 5µL full loop injection of 500 µg/mL budesonide EP grade (Spectrum Quality Products) standard solution. UPLC method conditions: ACQUITY BEH C_{18} 2.1 x 100 mm;1.7 µm, 68% 20 mM ammonium formate buffer to pH 3.2: 32% acetonitrile; wavelength at 240 nm; temperature at 40° C; flow rate at 0.6 mL/min; 11,500 psi. The additional peaks 1 thru 7 are identified as impurity peaks above 0.05% area.

Suitability Results

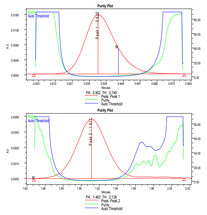
The %RSD of the sum of the areas of both epimers was 0.3% (n=6 injections) for the 500 µg/mL budesonide EP grade standard solution The results in the table below are for the budesonide EP 500 µg/mL standard solution. The requirements of the minimum European Pharmacopoeia specifications are met.

Name	Retention Time	Resolution	Symmetry Factor	Signal/ Noise	EP Plates
R— epimer	5.073	N/A	1.05	10262	17011
S— epimer	5.476	2.46	1.02	6646	17390

PDA Spectral Analysis (peak area % ≥ 0.05%)

Utilization of the PDA allows for testing peak homogeneity. Peak purity flags alert where co-elutions may occur. Peak purity plots can indicate spectral deviations at the front or tail of a peak where a co-elution can occur. Decisions can then be made to further develop the method to achieve separation. In this example, the flagged peaks indicate other unknown co-eluting impurities, however the area of the peaks in question are far below the EP individual peak related substance test specifications. Further method development was not performed to separate the possible co-elution. However, the MS data for each flagged peak will be analyzed for potential co-elutions that are not epimeric forms.

Name	RT (min)	% Area	λ max (nm)	Purity Flag (pass 1)
Peak 1	0.635	0.11	245.1	YES
Peak 2	1.911	0.06	245.1	No
Peak 3	2.085	0.19	240.2	YES
Peak 4	2.696	0.19	245.1	No
Peak 5	3.374	0.20	245.1	No
Peak 6	4.314	0.31	245.1	No
Peak 7	4.541	0.70	241.4	No
R—epimer	5.073	58.00	246.3	No
S—epimer	5.476	40.23	245.7	No



Impurity Profile Comparisons

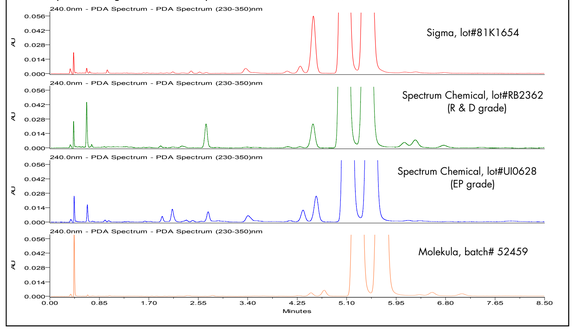
EP Related Substances Test

The EP related substances test as described in the Budesonide EP monograph was performed on four different batch lots of budesonide which were purchased from three different vendors. Test solutions (500 µg/mL) were prepared for each batch lot. Each test solution was diluted to yield two reference solutions each with concentrations of 2.5 µg/mL and 7.5 µg/mL with each representing 0.5% and 1.5% of the 500 µg/mL solution, respectively.

European Pharmacopoeia Related Substances Test Specification	Spectrum EP grade 98% - 102%	Sigma >99% purity	Molekula 100.2%	Spectrum Research grade (no spec)
Individual Impurities (x < 2.5µg/mL ∑ of epimers areas)	Fail	Fail	Pass	Fail
Total Impurities (x < 7.5µg/mL ∑ of epimers areas)	Fail	Fail	Pass	Fail
R—epimer/S—epimer Ratio (S—epimer is 40.0% to 51% ∑ of epimers areas)	59.24%/ 40.76%	50.49%/ 49.51	51.38%/ 48.62	58.66%/ 41.34
Purity	98.24%	97.99%	99.52%	98.07%

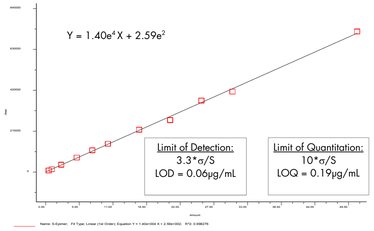
Impurity Profile Comparisons

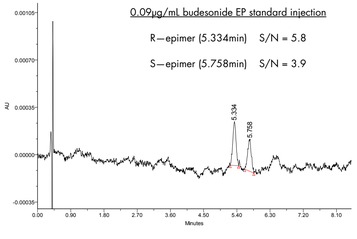
The impurity profiles for each lot was compared using the 500 µg/mL test solution. There were varied impurities detected and at different levels for each sample when prepared fresh. It was observed after exposure to light and time, the profiles demonstrated more similarities.



Method Sensitivity

A calibration curve constructed with triplicate injections of standard solutions prepared at 0.5 µg/mL to 50 µg/mL. The limit of detection was calculated using the linear regression equation for the S - epimer (the smaller epimer peak). For the LOD/LOQ, σ was the standard deviation of the y-intercept and S was the slope of the curve.

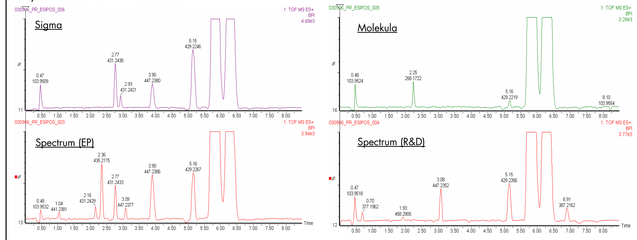




Exact Mass

MS Utility

Using LC/MS in purity profiling experiments aids in peak tracking during method development and facilitates a high level of confidence with known analyte identification when exact mass is employed. ACS requires < 5 ppm mass accuracy for patent submission and publication. The mass accuracy of the known peaks in each of the budesonide batches are less than 3 ppm (below) . By coupling exact mass with tools like elemental composition, it is possible to predict molecular formulas for the unknown analytes such as the 447.2380 amu.



Multiple MS peaks were found for masses such as 429 amu, 447 amu, and 431 amu in each of the batches. To compare the masses to the UV detected peaks in figure 1, the following was determined: Impurity A elutes as peak 1. The peaks with mass 431 (impurity C and epimer) elute as peaks 2 and 3. Peaks 4 and 5 have the unknown mass spectra of 447.2461 amu. This unknown could be some hydration of either impurity D or E (and epimer). The fragmentation has a spectral ion at 429 amu and 447 amu representing a difference of 18 amu (water). There are two peaks for 429 amu believed to be the epimeric compounds of impurity E that eluted as peaks 6 and 7.

Compound	[M+H]	Spectrum (EP)	Molekula	Sigma	Spectrum (R&D)
Known Impurity					
Impurity A	377.1964	✔		✔	✔
Impurity B and F	403.2120				
Impurity C	431.2433	✔	✔	✔	✔
Impurity D and E	429.2277	✔	✔	✔	✔
Impurity G	433.2512		✔		
Unknown Impurity					
1	447.2461	✔	✔	✔	✔
2	258.1722		✔		
3	435.2175	✔			

Table 3 : Each manufacturers' budesonide substance was analyzed by MS. The resulting table represents known and unknown impurities found in 500 µg/mL test solutions.

Conclusions

An Ultra Performance LC—MS method for budesonide and the related impurities was developed. European Pharmacopoeia specifications were met using the UPLC method. The resolution between the budesonide epimers was 2.5, EP plate count was 17,000, symmetry factor for the R—epimer was 1.05, and the area %RSD for six replicate injections of the 500 µg/mL test solution was 0.3%. These results far exceeds the EP specifications and any other published HPLC methodology. A linear regression was constructed from the calibration curve to determine the LOD and LOQ of the method. It was determined that the LOD was 0.06 µg/mL and the LOQ was 0.19 µg/mL. The developed method was used for impurity profile comparisons of four different budesonide batches. Peak purity was used to determine method completion while using MS for peak identification. The method proved to be very specific in determining the differences and similarities between each of the suppliers batches. Exact mass was performed to yield a mass accuracy below 3 ppm for each of the impurity peaks. An unknown mass of 447 amu was determined to be a hydration products of one of the impurities D/impurity E and the respective epimer. The system configuration was shown to be sensitive and accurate for the determination and identification of impurities related to pharmaceutical drug substances.

References

1. G. Roth, A. Wikby, L. Nilsson, A. Thalen, J. Pharm Sci. 69 (1980) 766—770
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3. S. Hou, M. Hindle, P.R. Byron, J. Pharm. Biomed. Anal. 24 (2001) 371—380

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