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A Novel Approach Utilizing MetaboLynx and MassFragment to Facilitate Impurity Profiling of Pharmaceuticals

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Abstract

In this application note, the analytical system yielded rapid identification and structural characterization of the impurities in a budesonide drug substance for peaks ≥ 0.05% relative total peak UV area; this was accomplished within a single analysis on one ACQUITY UPLC/SYNAPT MS instrument and one chromatographic data system for efficient data analysis.

Introduction

Impurity profiling of active pharmaceutical ingredients (API) is an essential part of drug development. Impurity profiling is not only critical to ensure the safety and quality of the API and the resulting drug product, but also enables a thorough understanding of the API's synthetic process and degradation pathway.

To achieve the high sensitivity and high resolution needed for the qualitative (0.5% area threshold) and quantitative (0.1% area threshold) analyses for impurities, scientists must employ a variety of technologies.

By employing UltraPerformance LC (UPLC) in combination with high sensitivity MS and automated method scouting, methods capable of detecting and resolving most of the impurities can be developed in a day or two. Enhanced resolution and sensitivity facilitates the confirmation of known or expected impurities. Now, the bottleneck becomes the elucidation of the unknown components that are observed.

Impurities originating from the drug substance most commonly originate during the synthetic process or degradation. In this application note, we will apply software tools to process a UPLC-MS^E dataset for characterization of an impurity profile related to a pharmaceutical active drug substance.

Budesonide is a glucocorticosteriod used for the treatment of asthma via various matrices and inhalation mechanisms. The stereochemistry of this compound adds a common challenge that chemists face with impurity profiling, therefore mass spectrometry plays a central role in our approach rather than the use of only UV detection.

With information about predictive pathways and process and degradation components of the API, the software tools enable characterization of impurities with higher throughput and confidence, thus reducing the bottlenecks associated with impurity profiling.

Experimental

In MS^E acquisitions (Figure1), low and high collision energy data are acquired in parallel in a single injection. The low and high energy data are stored within two separate functions of a single data file.

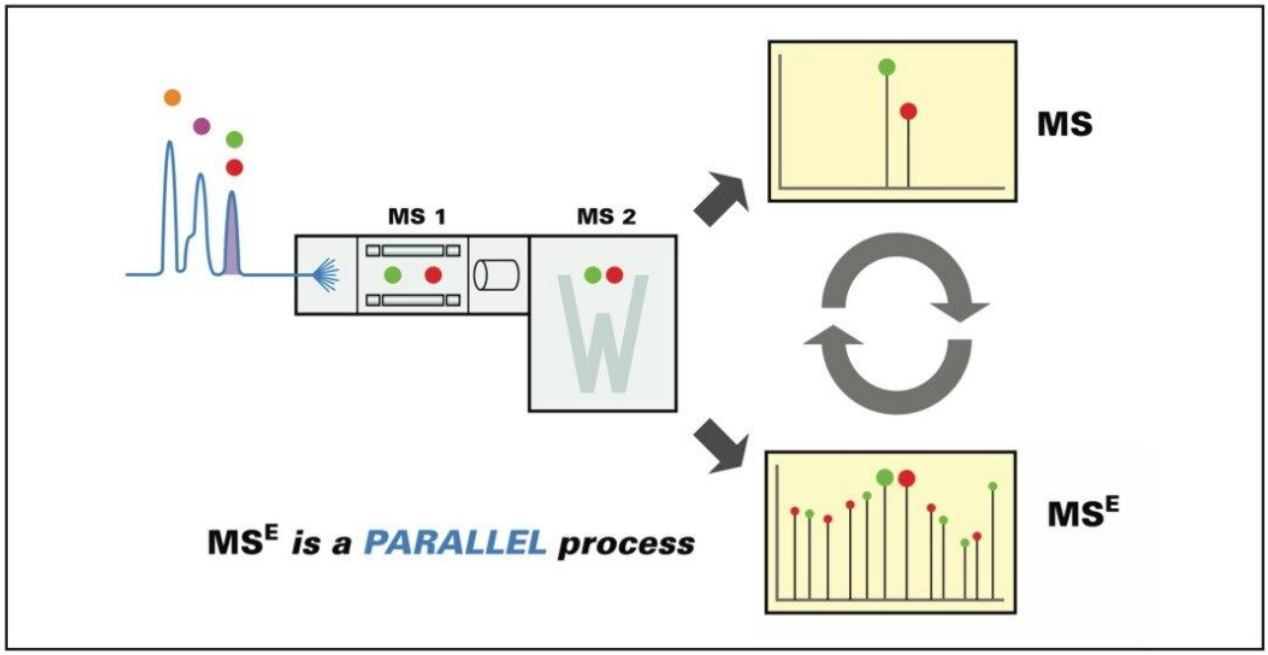

Figure 1. MS^E acquires low and high collision energy data simultaneously.

Data was processed using MetaboLynx, an application manager within MassLynx Software that enables automatic identification of sample components using exact mass measurement and elemental composition data. Routinely applied to *in vitro* and *in vivo* metabolite profiling studies, this software can also be applied to impurity analysis, allowing the analyst to quickly and confidently identify known and unknown impurities within a pharmaceutical compound.

LC Conditions

LC system:	Waters ACQUITY UPLC System
Column:	ACQUITY UPLC BEH C_{18}

	Column 2.1 x 100 mm, 1.7 µm
Column temp.:	40 °C
Flow Rate:	600 µL/min
Mobile phase A:	68% 20 mM Amm. Formate, pH 3.6
Mobile phase B:	32% Acetonitrile
Injection Volume:	5 µL
Wavelength:	240 nm

MS Conditions

MS system:	Waters SYNAPT MS System
Ionization mode:	ESI Positive
Capillary voltage:	3000 mV
Cone voltage:	20 V
Desolvation temp.:	350 °C
Desolvation gas:	800 L/Hr
Source temp.:	120 °C
Acquisition range:	100 to 1000 *m/z*
Collision Energies:	High 30 eV, Low 4 eV

Scan Time:	0.095 s
Interscan delay:	0.020 s
Lock mass:	Leucine/Enkephalin 500pg/µL @ 50µL/min

Results and Discussion

Identified impurities detected within the budesonide sample in the low energy MS^E function are displayed in the MetaboLynx browser and recorded in the searchable impurity list (Figure 2).

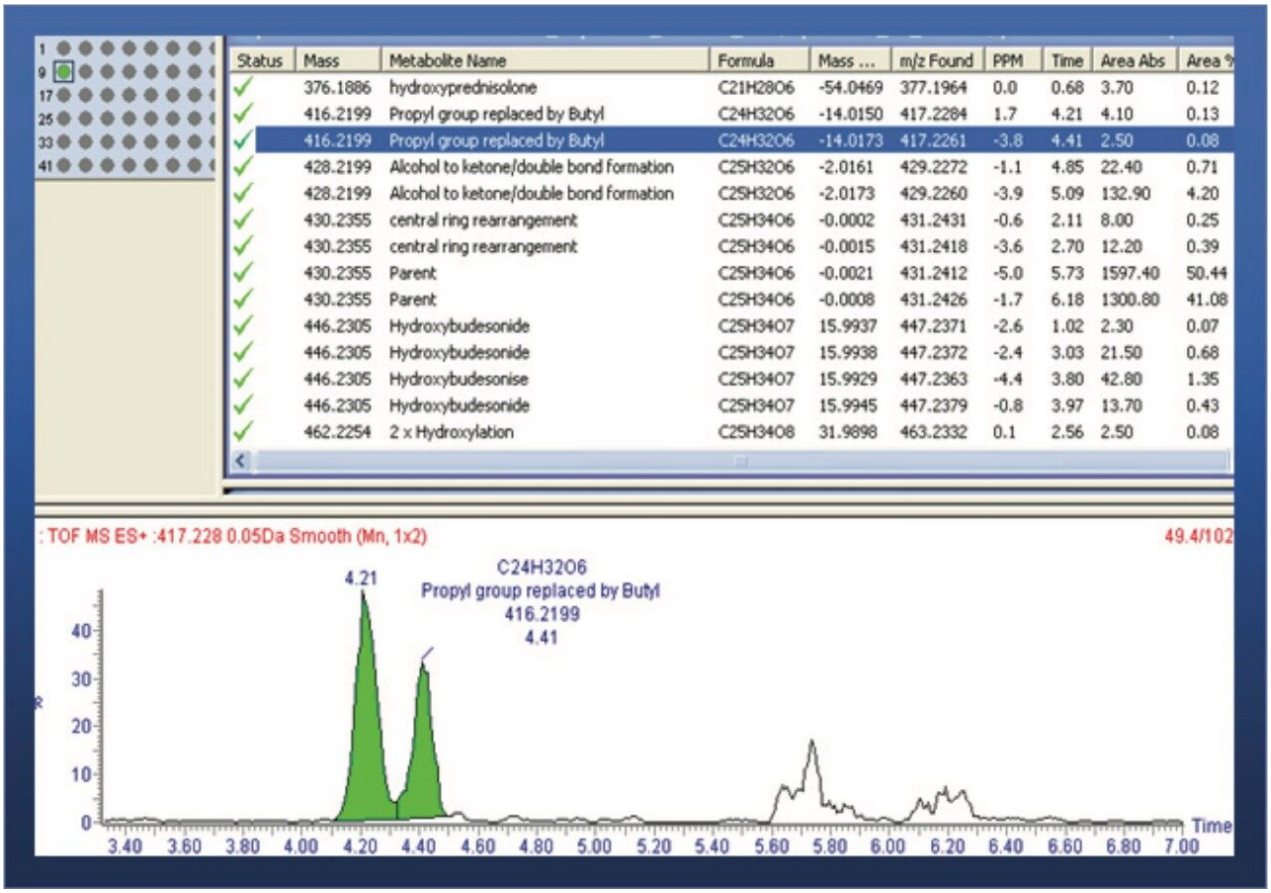

Figure 2. The MetaboLynx Application Manager browser.

Twelve impurity peaks were flagged, comprising five known impurities and seven previously unknown impurities. The overall mass error for all impurities identified was 1.7 ppm RMS, hence filtering down the number of possibilities given by elemental composition. Two resolved peaks were observed for impurities containing the same chiral center as budesonide. This information was additional confirmation of the impurity assignment by MetaboLynx.

Fragment ion information from the high energy MS^E function was used for full structural identification of some impurities.

MetaboLynx MS^E Fragmentation Analysis

The software algorithm MetaboLynx MS^E was used to mine both high and low collision energy scans simultaneously. This enables the visual alignment of precursor with collision-induced dissociation (CID) fragment ions for budesonide and its impurities (Figure 3).

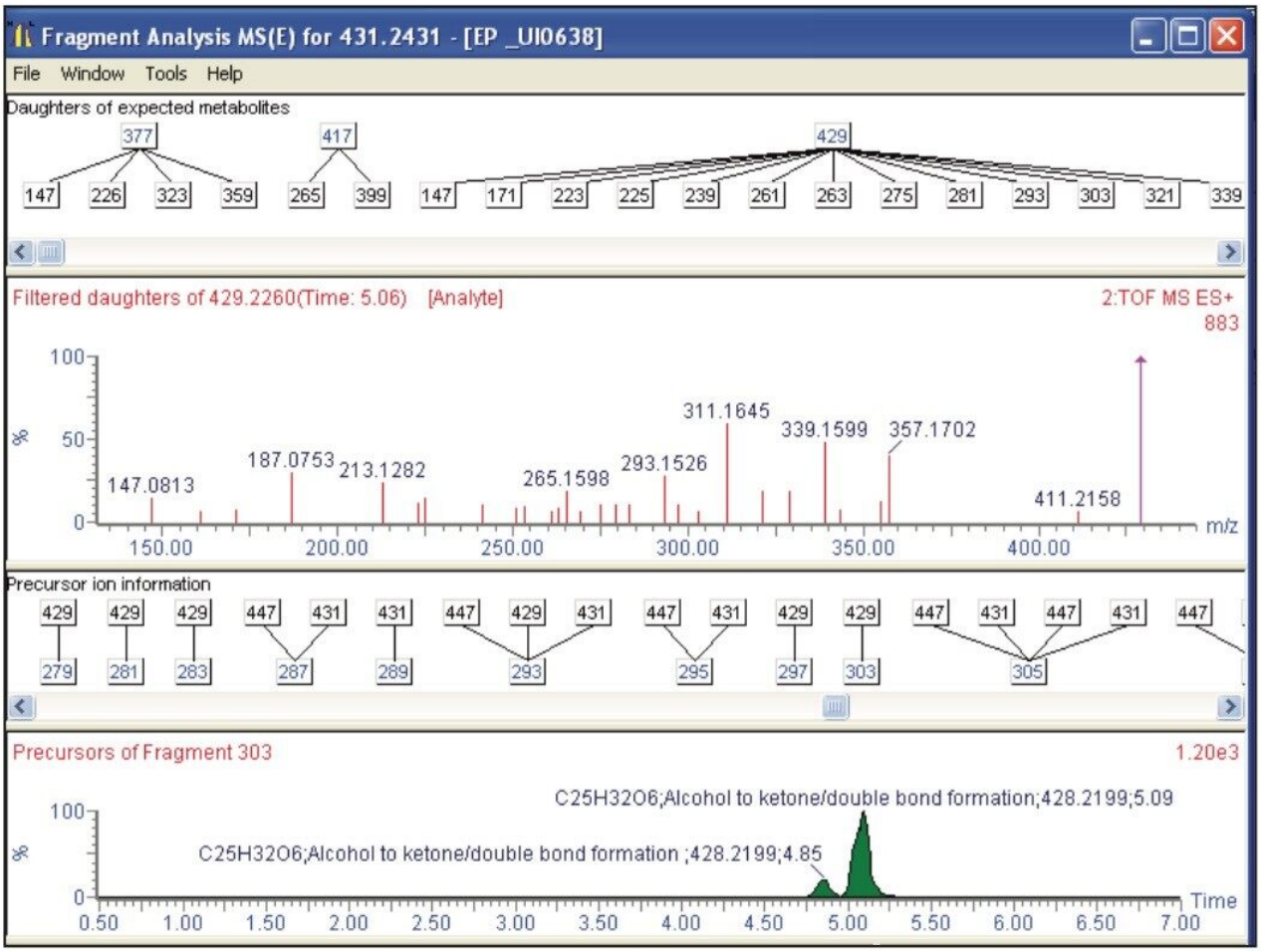

Figure 3. MetaboLynx fragment analysis of Budesonide and impurities.

MassFragment Tool

The automated structure elucidation tool, MassFragment, was employed to rationalize and identify fragment ion structures. Using exact-mass high energy MS^E data, with a systematic bond disconnection algorithm and a ranking principle, the structures of the fragment ions of budesonide and its impurities were rapidly assigned (Figure 4).

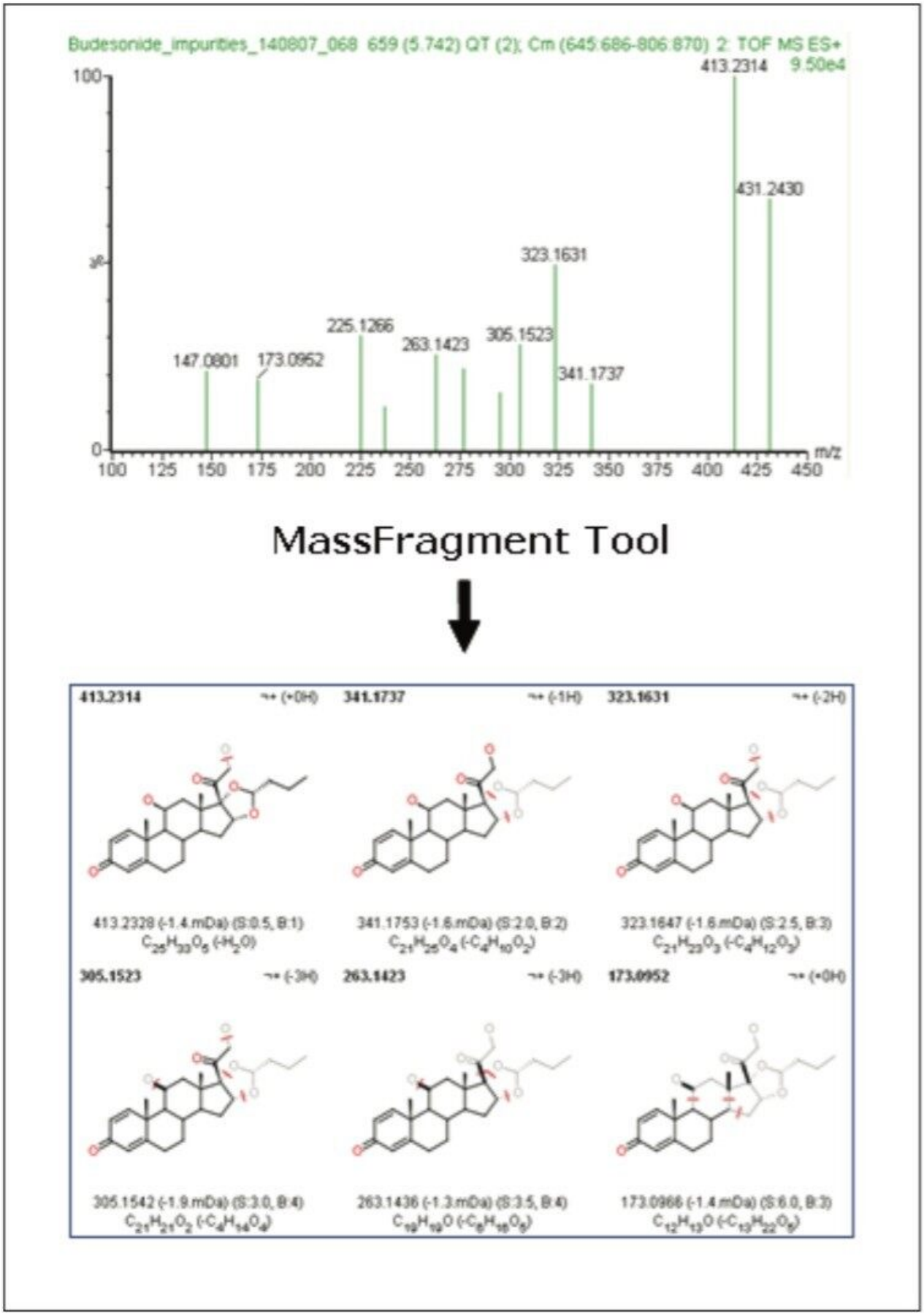

Figure 4. MassFragment assignment of the fragment ions of budesonide.

The complementary information obtained from the MetaboLynx MS^E

fragmentation analysis and the MassFragment structure elucidation tool enabled rapid structural confirmation of impurity peaks.

Budesonide and all detected impurities were found to have a common fragment ion with *m/z* 173.0961 that is consistent with cleavage of rings C and D of the protonated molecule, as shown in Figure 5. This suggests that rings A and B of the budesonide structure are unchanged in all the detected impurity molecular ions.

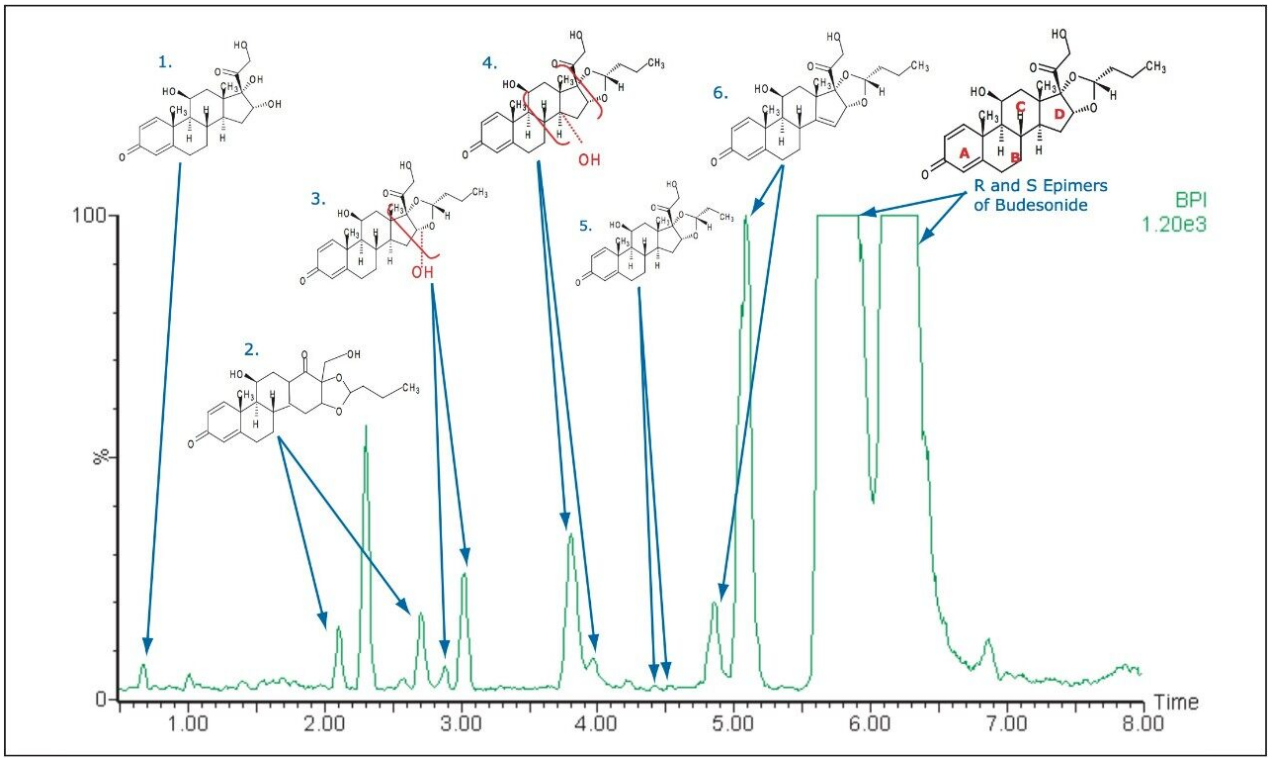

Figure 5. MS chromatogram with peak-to-structure assignments.

Impurity 4, identified as hydroxylated budesonide, gave fragments ions at *m/z* 339.1157 and 311.1682. These masses are consistent with the addition of oxygen to rings C and D of budesonide. The fragment ion with *m/z* 305.1523 common to budesonide and several impurities (see above) was not observed for impurity 6. The presence of a fragment ion with *m/z* 303.1307 for this impurity was suggestive of a double bond within ring D of Budesonide.

Conclusion

The use of mass spectrometry in impurity profiling enables detection of very low levels of impurities and provides assistance with structural characterization of these compounds.

The ability to acquire accurate mass and collision-induced fragmentation generally requires both time-of-flight instrumentation and tandem quadrupole mass spectrometry. Even when using tandem quadrupole MS, the analyst would traditionally need multiple methods to acquire both low collision energy precursor ion data and high collision energy product ion data. Correlation of the data generated from each instrument further complicates evaluation of the results.

Using SYNAPT MS in the MS^E mode, all of the information needed to identify components in an impurity profile can now be generated in a single UPLC injection. MetaboLynx and MassFragment Software tools streamline data analysis and automatically provide component identification and structural characterization.

In this example, the analytical system yielded rapid identification and structural characterization of the impurities in a Budesonide drug substance for peaks ≥ 0.05% relative total peak UV area; this was accomplished within a single analysis on one ACQUITY UPLC/SYNAPT MS instrument and one chromatographic data system for efficient data analysis.

Manual data evaluation of the exact mass values, MS/MS fragmentation of the impurities, elucidation of unknowns and correlation of the impurities to the API and/or synthetic process normally takes approximately one to two weeks of evaluation time, and two to three days of instrument preparation and analysis time.

The use of ACQUITY UPLC/SYNAPT MS and MS^E with MetaboLynx and MassFragment reduced the time needed to identify and characterize the impurities in budesonide drug substance to about one or two days total, while allowing more time for more targeted MS/MS as necessary.

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ACQUITY UPLC System <https://www.waters.com/514207>

MetaboLynx XS <https://www.waters.com/513803>

MassFragment <https://www.waters.com/1000943>

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