AN AUTOMATED APPROACH FOR PHARMACEUTICAL IMPURITY PROFILING

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INTRODUCTION

Impurity profiling of pharmaceutical active ingredients is an essential part of the research and developmental cycle. Regulatory agencies consider impurity profiling critical to ensure the safety and efficacy of the pharmaceutical ingredients through the practice of "good science" prior to filing. Scientists need to employ a variety of technology and experience to develop methods with high sensitivity and high resolution in such to provide qualitative and semi-quantitative information on all peaks present above 0.05% area thresholds (qualitative) and 0.1% area thresholds (quantitative). Automated method scouting with ultra-performance chromatography has alleviated the time consuming process of resolving and detecting many of the impurities. However, the bottleneck now becomes the confirmation and elucidation of the known and unknown components that are observed.

Impurities originating from the drug substance most commonly originate within the synthetic process or degradation. In this presentation, we will apply various software tools to process a UPLC/MS^E data set for characterisation of an impurity profile related to a pharmaceutical active drug substance; Budesonide. 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, hence why mass spectrometry plays a central role in our approach rather than the use of only ultraviolet detection. With information about predictive pathways and, process and degradation components of the API, the software tools enable characterisation of impurities with higher throughput and confidence, thus reducing the bottlenecks associated with impurity profiling.

MS^E ACQUISITION

In MS^E acquisitions (Fig.1) low and high collision energy data is acquired in parallel in a single injection. The low and high energy data is stored within 2 separate functions of a single data file

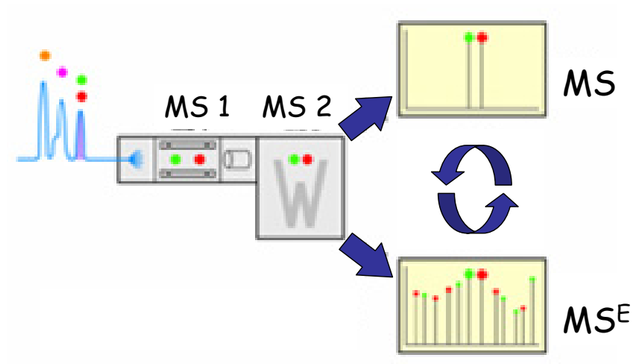


Fig.1 MS^E acquires low and high collision energy data simultaneously

Experimental

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^{0}C
\injection Volume:	5 µL
Detection:	ACQUITY PDA @ 240 nm

MS Conditions

Instrument:	Waters® Q-ToF Premier™
Software:	Masslynx™ 4.1

Tune Page Parameters:

Source:	ES+
Capillary (V):	3.0
Sample Cone (V):	57 for reference 20 for analyte
Extraction Cone (V):	4.5
Desolvation Temp (^{0}C):	350.0
Source Temp (^{0}C):	120.0
Desolvation Flow (L/Hr):	800.0

Tof Settings

Acquisition Range:	100 - 1000 Da
Scan Time:	0.095s
Interscan delay:	0.020s
Lock mass:	500pg/µL Leucine/Enkephalin @ 50µL/min

MS^E settings

Low collision energy:	4eV
High collision energy:	30eV

AUTOMATED IMPURITY IDENTIFICATION

Data was processed using Metabolynx™, an application manager within MassLynx™ software, which enables automatic identification of sample components using exact mass measurement and elemental compositions data. Routinely applied for *in vitro* and *in vivo* metabolite profiling, 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.

RESULTS

The impurities detected within the Budesonide sample in the Low energy MS^E function are displayed in the Metabolynx browser (Fig.2).

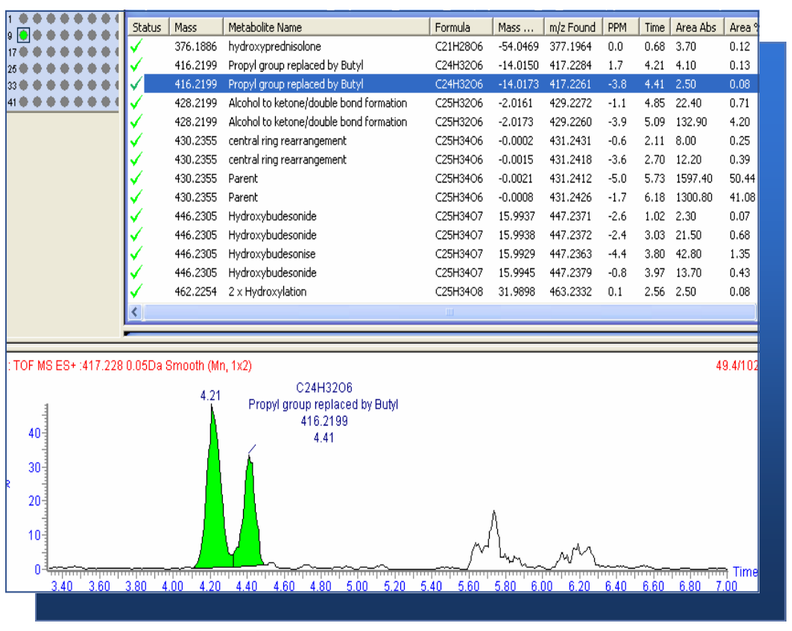


Fig.2 Metabolynx browser

- 12 impurity peaks were flagged, comprising 5 known impurities and 7 previously unknown impurities .
- The overall mass error for all impurities identified was 1.7ppm RMS
- Impurities were detected to 0.05% of total peak area
- 2 resolved peaks were observed for impurities containing the same chiral centre as Budesonide. This information acted as additional confirmation of impurity assignment by Metabolynx

Fragment ion information from the high energy MS^E function was required 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 collisions energy scans simultaneously. This enables the visual alignment of precursor with CID fragment ions for Budesonide and its impurities (Fig 3).

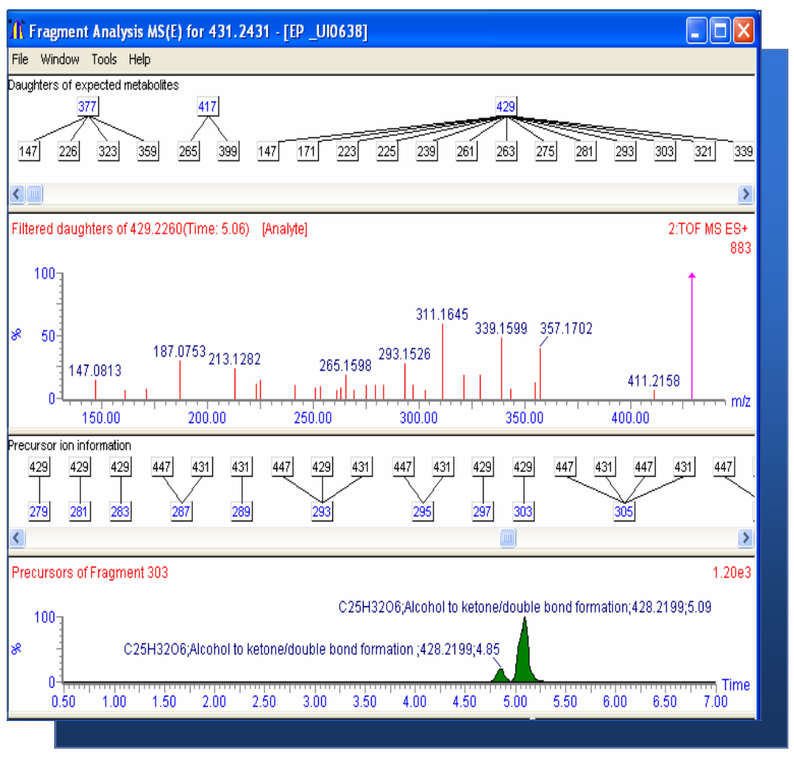
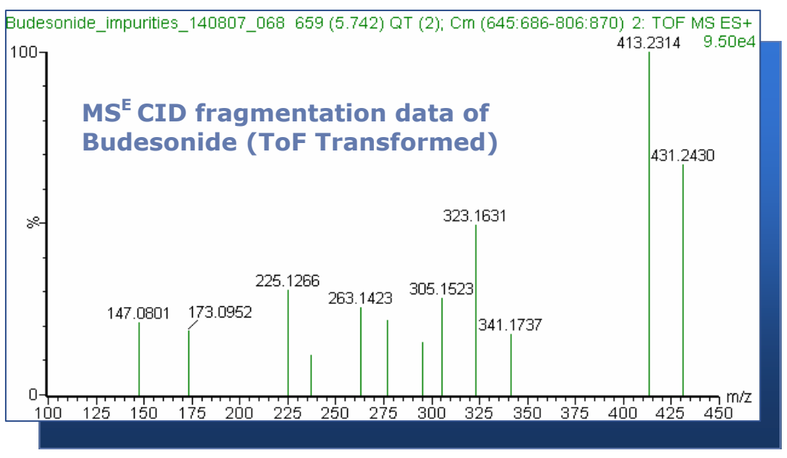


Fig 3. Metabolynx fragment analysis of Budesonide and Impurities

MASSFRAGMENT™ TOOL

The automated structure elucidation tool, MassFragment™, was employed to rationalise 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 (Fig 4)



Mass Fragment Tool

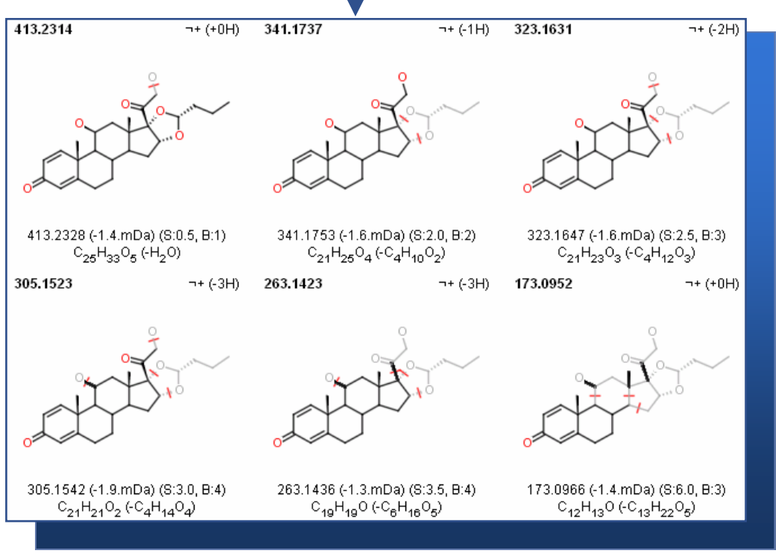


Fig.4 Mass Fragment assignment of the fragment ions of Budesonide.

The complementary information obtained from the Metabolynx MS^E fragmentation analysis and the Mass Fragment structure elucidation tool enabled rapid structural confirmation of impurities peaks.

Fragmentation Explained;

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

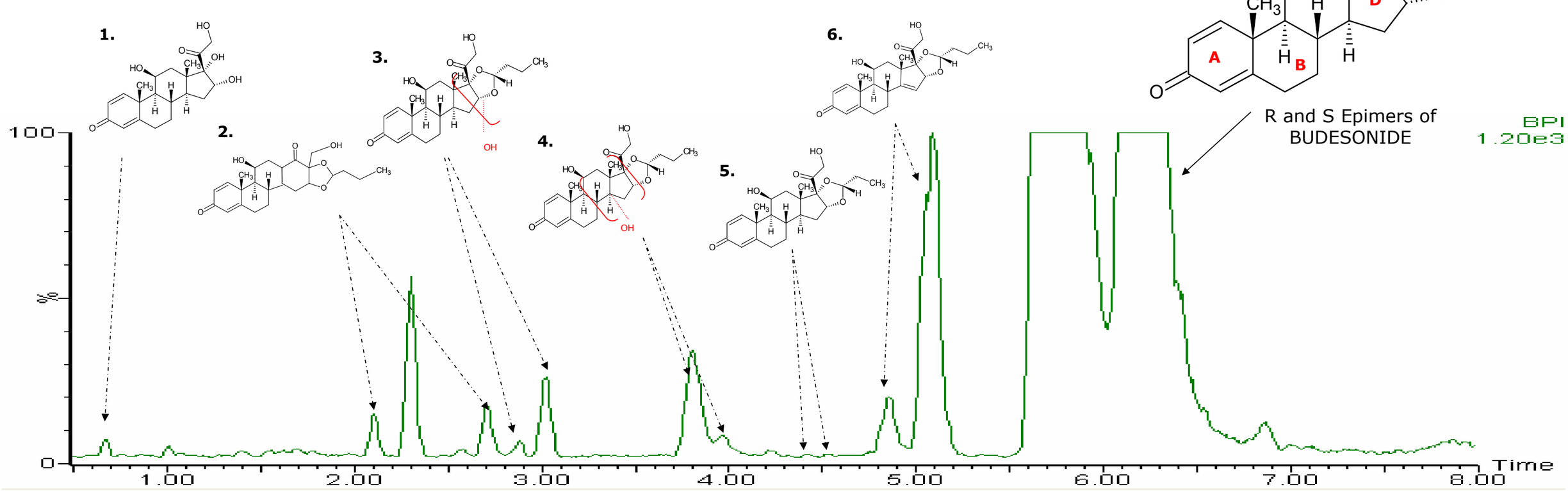
Impurity **4.**, dentified as hydroxylated Budesonide, gave fragments ions at *m/z* 339.1157 and 311.1682. These masses are consistent with the addition of oxygen to rings C and D of Budesonide .

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.

CONCLUSIONS

The use of Mass Spectrometry in impurity profiling is important not only due to the ability to detect very low levels impurities but also the opportunity for structural characterisation of these compounds . Metabolynx UPLC/MS^E and the complementary software tool, Mass Fragment, offer an automated approach to processing the rich structural information gained from exact mass data. In this example, this analytical system enabled rapid identification and structural characterisation of the impurities in a Budesonide reference standard (≥ 0.05% relative total peak area), within a single analysis.

BUDESONIDE IMPURITY PROFILE PEAK ASSIGNMENT



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