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Employing UPLC-UV-ToF MS^E and the UNIFI Scientific Information System for the Analysis of the Forced Degradation of Glipizide

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APPLICATION BENEFITS

- Comprehensive impurity analysis using the UNIFI® Scientific Information System.
- Integration of the PDA and MS detectors, combined with trend plots and custom calculations for a complete and comprehensive high resolution impurity analysis solution.
- Simultaneous collection of accurate mass precursor and fragment ion data without the need for preselection of precursors (MS^E).

WATERS SOLUTIONS

ACQUITY UPLC® I-Class System

Xevo® G2-XS QTof

ACQUITY UPLC BEH C_{18} Column

UNIFI Scientific Information System

KEYWORDS

Impurity analysis, glipizide, high resolution mass spectrometry, forced degradation

INTRODUCTION

UltraPerformance Liquid Chromatography (UPLC®), photo diode array (PDA) detection characterisation of both known and unknown impurities is a critical step in the pharmaceutical industry.[1,2] There is a need to more rapidly identify and further characterise these impurities. By employing high resolution mass spectrometry (HRMS) techniques we can establish a comprehensive dual detection and characterization strategy.

A tightly integrated data package for the acquisition, identification, confirmation, interrogation, reporting, and storing information in libraries provides a powerful approach for impurity profiling, and has proven to be exceptionally beneficial to scientists for the characterization of complex samples.

A comprehensive precursor and product ion dataset acquired by use of the MS^E acquisition mode is demonstrated. MS^E simultaneously acquires full scan precursor and product ion spectra from a single acquisition without the requirement to preselect precursor ions. This simplifies data acquisition because it does not require advanced knowledge of the analytes.[3] Additionally, it also ensures all ionizable impurities can be detected, and in most cases identified, in the first acquisition.

In this technology brief, glipizide was subjected to accelerated stress conditions to generate degradation products.[4] The resulting impurities were identified, interrogated and visualised using the UNIFI Scientific Information System.

EXPERIMENTAL

Sample description

Acid and temperature degradation: The acid catalyzed/temperature degradation studies were carried out by adding a small aliquot of formic acid to a solution of glipizide. The sample was then left at 80 °C and aliquots taken at 0, 6, 24, 48, 72, and 96 hour time points. The samples were diluted 1:10 with mobile phase prior to injection.

Method conditions

System:	ACQUITY UPLC I-Class (FTN)
Column:	ACQUITY UPLC BEH C_{18}, 2.1 x 150 mm, 1.7 µm
Run time:	11.0 minutes
Vials:	Waters Maximum Recovery
Column temp.:	45 °C
Sample temp.:	8 °C
Injection volume:	0.5 µL
Flow rate:	0.4 mL/min
Mobile phase A:	water + 0.1% formic acid
Mobile phase B:	acetonitrile + 0.1% formic acid

Gradient:

Time	%A	%B	Curve
0.0	95	5	–
7.0	40	60	6
8.0	0.0	100	6
9.0	95	5	11

MS^E conditions

MS system:	Xevo G2-XS QTof
Ionization mode:	ESI+
Source temp.:	120 °C
Desolvation temp.:	450 °C
Desolvation gas:	800 L/hr
Reference mass:	Leucine enkephalin $[M+H]^+$ *m/z* 556.27658
Acquisition range:	*m/z* 50–1200
Scan time:	0.1 sec
Capillary voltage:	1.0 kV
Cone voltage:	25 V
Collision energy:	Function 1: 6 eV Function 2: Ramped 25–45 eV

Data management

Pathway profiling – UNIFI v1.8.2

RESULTS AND DISCUSSION

In this study, glipizide (shown in Figure 1) was used as a model compound to demonstrate impurity identification using UPLC-MS^E and the UNIFI Scientific Information System.[5,6] Two main impurities at *m/z* 379.1074 and *m/z* 321.1020 were identified and have also been reported in the literature.[4] Using accurate *m/z* values, UNIFI determined and verified the correct molecular formulas $C_{16}H_{18}N_4O_5S$ and $C_{14}H_{16}N_4O_3S$, respectively.

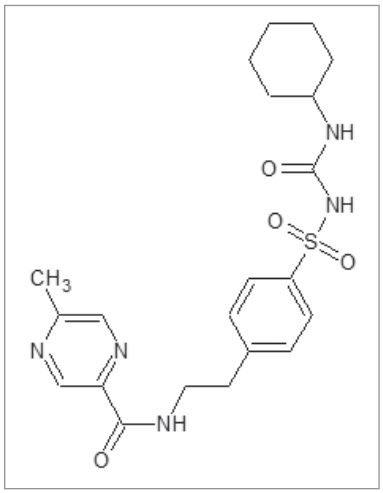

Figure 1. Glipizide.

Glipizide and the two impurities identified in the samples by mass, isotopic intensity and isotopic mass are tabulated along with a trend plot (Figure 2). The trend plot details the decrease of glipizide followed by the concomitant riseof the impurities.

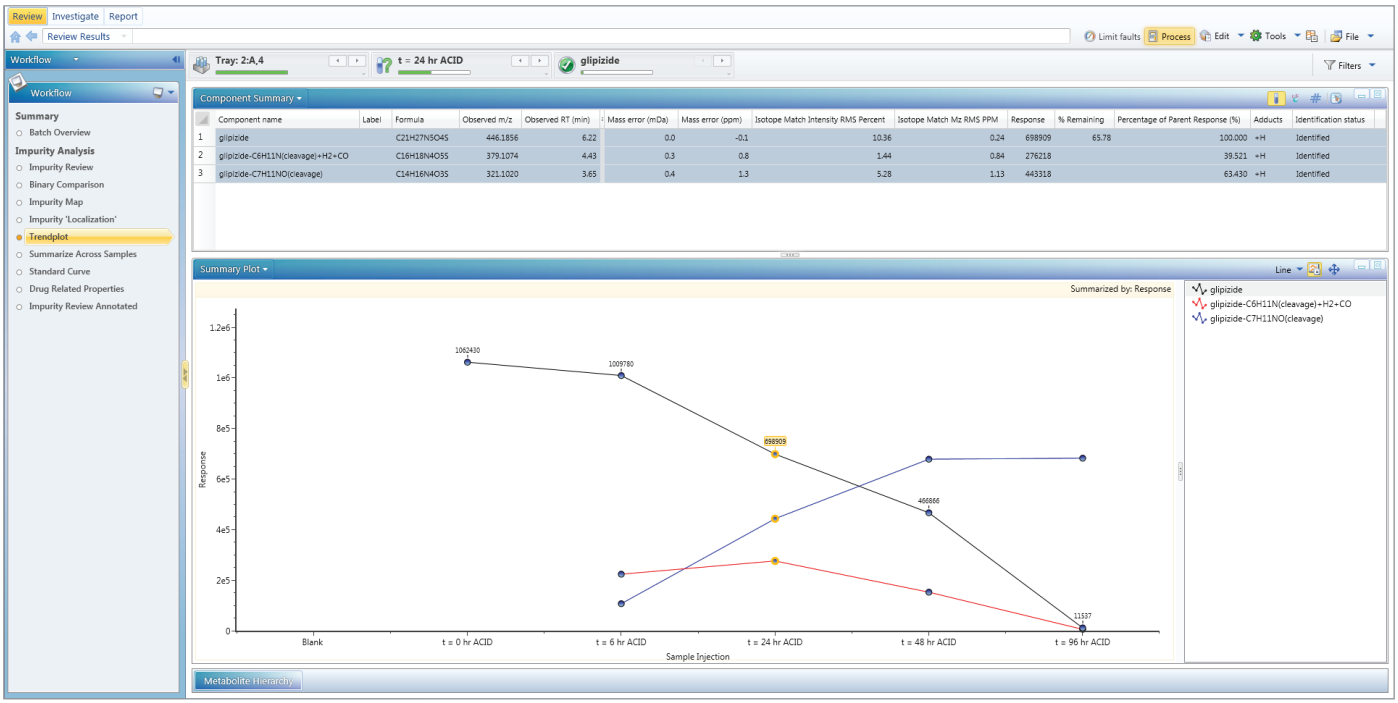

Figure 2. Glipizide and the identified impurities with their response plotted over the experimental time course.

As depicted in Figure 3, the spectrum shows the low energy channel containing precursor ion information and the bottom spectrum shows the high energy MS^E channel, containing fragment ion information. Product ion assignment is automatically performed during the analysis, annotated directly on the spectrum, greatly simplifying interpretation. In the high energy data, the ion at *m/z* 321.1019 has been annotated showing the bond cleavage and the charge retaining portion of the molecule.

Figure 3. Low and high energy (MS^E) spectra and UV plot reported. The fragment ions are automatically assigned based on the structure (.mol file) of the API.

A custom calculation was used in order to show the % remaining (or conversion of glipizide) over the course of the experiment (Figure 4). The extracted ion chromatogram (XIC) of glipizide in the 24 hr sample (bottom left panel of Figure 4), as well as the PDA trace at 254 nm and a summed trace of all the identified components, is displayed in the component table representing data from all of the injections.

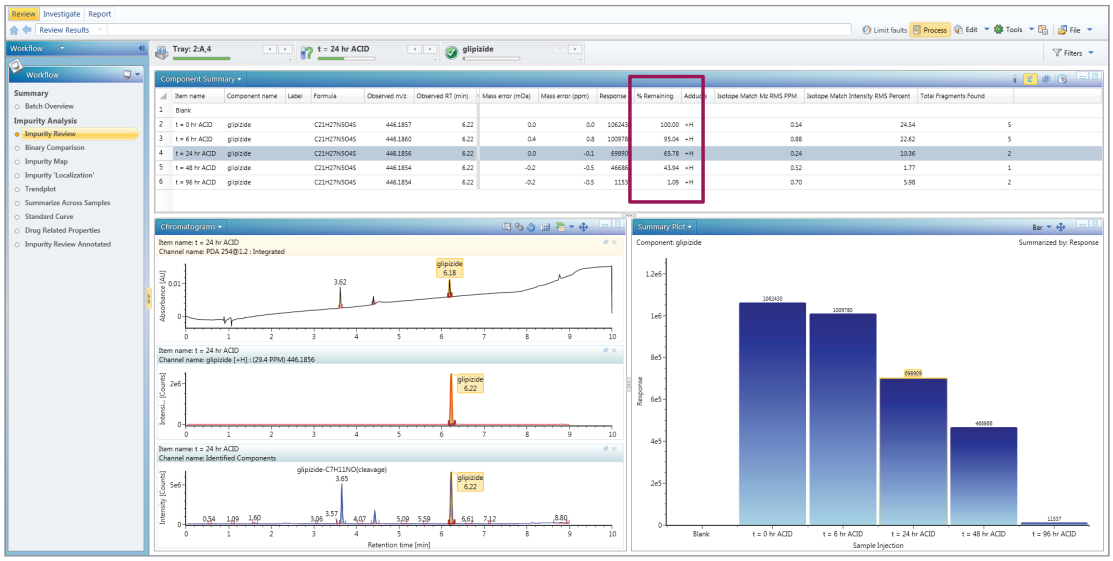

Figure 4. Response and percentage remaining of glipizide over the experimental time course.

For further characterization, it is possible using the UV or MS, to determine the relative response of the API and any impurities identified within the samples. Custom calculations were implemented (Figures 5 and 6) displaying the percentage of glipizide against the two impurities within the 48 hr sample, respectively.

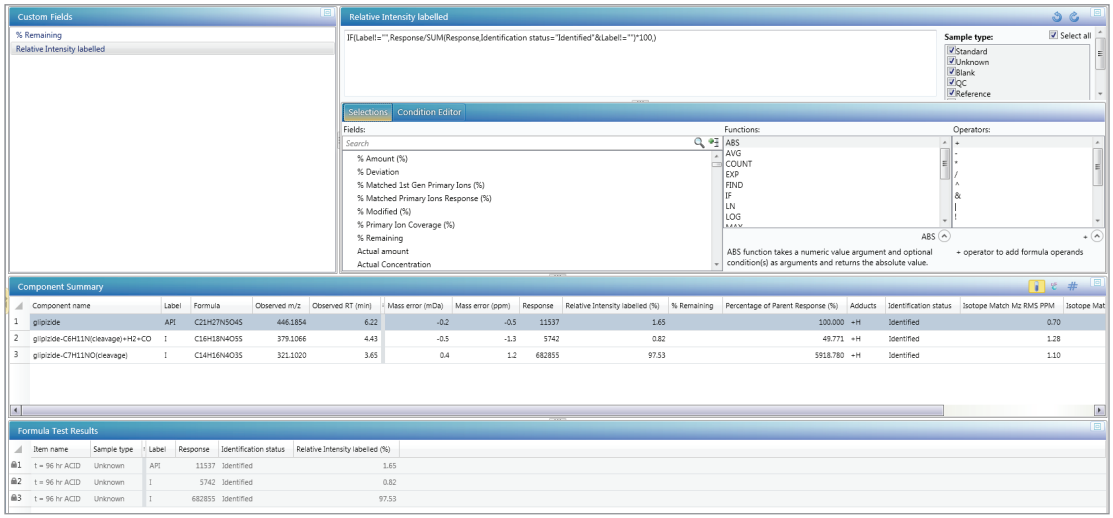

Figure 5. Building a custom calculation within the editor for calculating the relative intensity of the API and identified impurities within a sample. This can be based on MS or UV response.

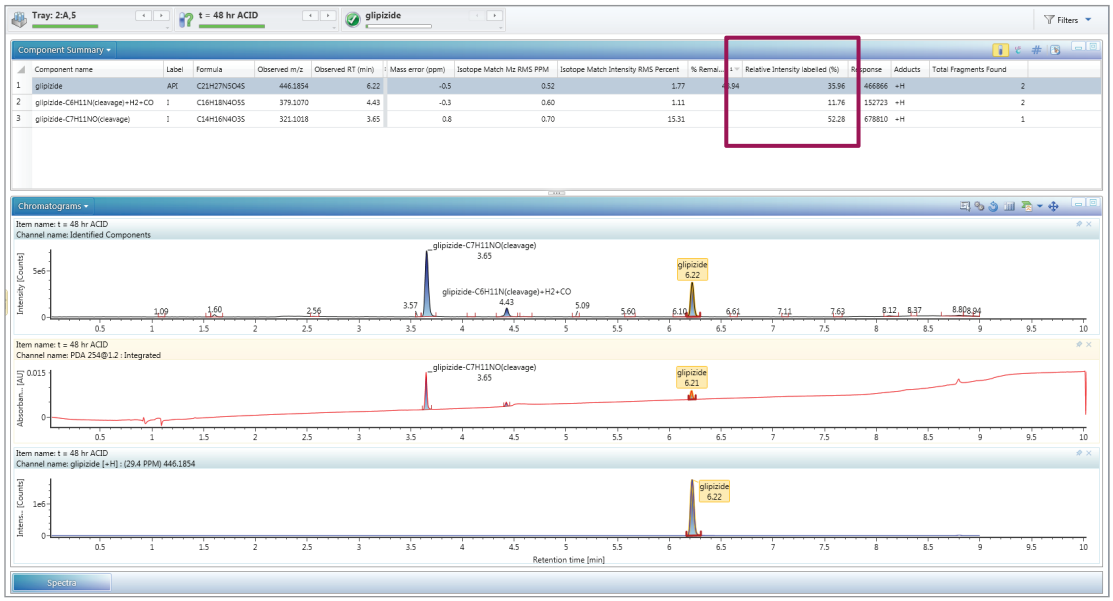

Figure 6. Use of a custom calculation displaying the relative intensity of glipizide and the impurities within a sample.

CONCLUSIONS

Forced degradation and impurity identification of glipizide was successfully characterized utilizing UPLC with PDA and HRMS detection on the UNIFI Scientific Information System platform. The ability to acquire accurate MS^E data within one injection allowed glipizide and its impurities to be rapidly identified and comprehensively confirmed. UNIFI enabled the use of custom calculations, incorporation of UV (and analogue) traces as well as trend plots to easy visualize and interpret their relationship and stabilities.

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