DEMONSTRATING AN EASE-OF-USE BENCHTOP TIME OF FLIGHT DETECTOR AS AN EASE-OF-USE SOLUTION FOR ACCURATE MASS MEASUREMENTS IN FORCED DEGRADATION STUDIES

Authors Chris Henry[1](christopher_henry@waters.com), Paul Rainville[2]
Affiliations: [1]Waters Corporation, Stamford Avenue. Altrincham Road, Wilmslow, Cheshire, SK9 4AX, UK
[2] Waters Corporation, 34 Maple Street, Milford, MA 01757, US

INTRODUCTION

Forced degradation is a critical analytical study for the development of stability-indicating methods used by pharmaceutical companies as part of regulatory submissions to the FDA[1,2]. High Resolution Mass Spectrometry(HRMS) is a technique often used to identify chemical components in complex mixtures, but requires skilled users for operation and data interpretation. An ease-of-use benchtop Time of Flight (ToF) mass analyser, the Waters ACQUITY™ RDa detector was recently introduced to facilitate the deployment of accurate mass measurement technology, incorporating routine workflows and removing the need for HRMS expertise.

To demonstrate this the anti-diabetic drug glipizide was exposed to acidic, basic and oxidative conditions and submitted for analysis using the conditions described.

Within the UNIFI controlling software, a workflow would involve creating a library of known degradants and using that library to screen the data collected for any degradants that have actually been formed during the incubations.

In this example, theprocessing method within UNIFI we have imported mol files of Glipizide API (Active Pharmaceutical Ingredient) and 5 known impurities . UNIFI used this information to interrogate the data generated and intelligently assign identified compounds and fragments.

Sample Preparation.

Img/mL of glipizide standard was dissolved in methanol . A 0.5 mL aliquot of this solution was transferred to reaction vials and chemically stressed using formic acid, sodium hydroxide and hydrogen peroxide @ 80°C. Samples of each stress condition were removed from the heat at various timepoints, cooled and a 100µL aliquot taken and diluted in 95:5 water : methanol to a concentration of 100µL/mL. The samples were then analysed under the conditions detailed below.

LC Conditions

LC System:	ACQUITY UPLC I-Class PLUS
Detection:	ACQUITY TUV
Vials:	TruView Max Recovery Vials, PN186005668CV
Column(s):	ACQUITY BEH C18 2.1 x 100mm, 1.7µm
Column Temp.:	45°C
Sample Temp.:	8
Injection Volume:	1µl
Flow Rate:	0.4mL/min
Mobile Phase A:	Water/ 0.1% Formic Acid
Mobile Phase B:	Acetonitrile / 0.1% Formic Acid
Gradient:	5% B to 100% B, (8 minutes)

MS Conditions

MS System:	ACQUITY RDa Detector
Ionization Mode:	ESI +
Acquisition Range:	100-2000 Da
Capillary Voltage:	1.5kV (default)
Cone Voltage:	30V
Fragmentation Cone Voltage Ramp	60 – 150V
Scan Rate	10Hz
Desolvation Gas Temp	550°C

The ACQUITY RDa was operated at default acquisition range of 100-2000Da and desolvation temp of 550°C with capillary voltage of 1.5kV. A cone voltage of 30V was employed with a cone voltage ramping referred to as 'full scan with fragmentation function' set at 60-150V. Full scan mode with fragmentation function allows for the simultaneous acquisition of both high and low energy spectra. The automatically assigned fragmentation information provided further confidence for compound identification.

The LC/UV chromatogram was also acquired, using a Waters ACQUITY TUV @254nm, for additional information.

UNIFI processing Method and Workflow

As part of the UNIFI™ software workflow, MOL. files of the glipizide API and its associated impurities (*Figure 1*) were imported into the processing method. Using this information UNIFI screened the acquired data, and intelligently assigned any compounds and fragments detected.

Using a modifiable step-by-step workflow the data can be reviewed as required. Using the ' binarycompare' function a reference sample, can be compared to degraded sample and the profiles examined for peaks generated from degradation

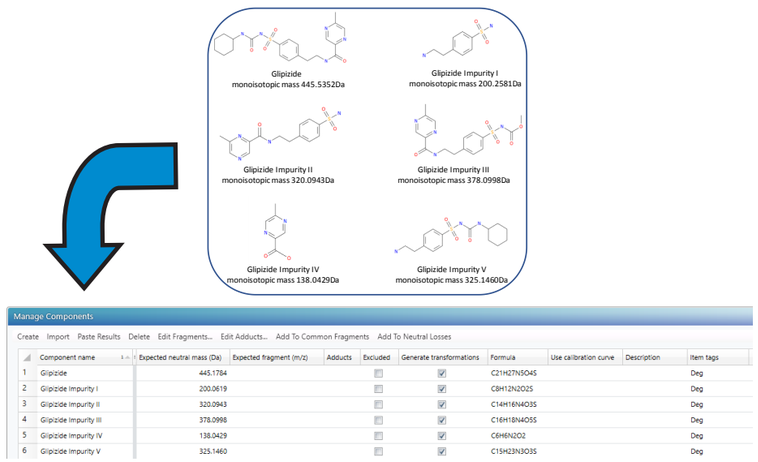

Figure 1. Component table in UNIFI with incorporated Mol. files of the API glipizide and five associated impurities

RESULTS

Binary Compare

Figure 2. 'Binary Compare' feature in UNIFI showing a reference spectra and a sample of glipizide degraded under acidic conditions for 60mins. The top comparison shows glipizide (@ 4.32mins highlighted in blue) present in both samples with structural visualisation as confirmation. The bottom spectra has a peak at 3.21 minutes .(highlighted in orange) which UNIFI has identified and visualised as "impurity III".

Impurity Profile

Figure3. Top: Acid degraded glipizide with 'impurity profile' selected in the workflow (1), main review pane (2) showing UV and XIC of impurity II and III. Impurity III is selected showing high and low energy acquisitions (4) with structural visualisation of the impurity and an associated fragment. Middle: Basic degradation with increase of impurity V plotted in the summary plot (4).Bottom: Oxidative degradation with line graph overlay of glipizide and concomitant generation of impurities II and III over time.

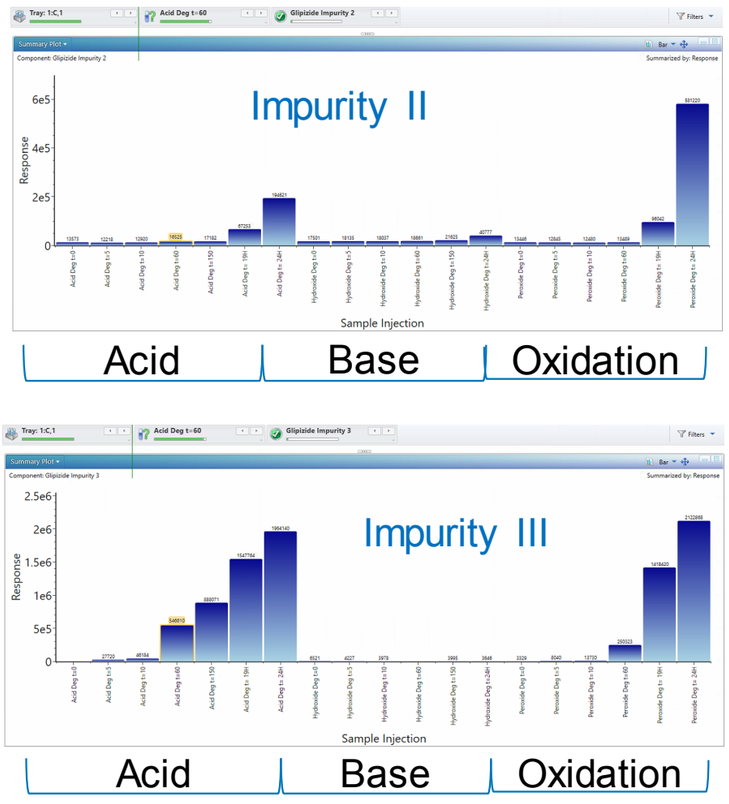

Figure 4. Summary profile of (Top) ; Impurity II present in the acid degraded samples and to greater extent in oxidative conditions.
(Middle); Impurity III readily formed in acidic and oxidative conditions and (bottom) Impurity V formed only under basic conditions.

DISCUSSION

The ACQUITY RDa Detector, coupled to the ACQUITY UPLC I-Class PLUS successfully separated, identified and characterised Glipizide and the resultant degradants from stressing under acidic, basic and oxidative conditions.

Results showed that glipizide stressing under acidic and oxidative conditions generated impurities 2 and 3 with the latter being produced more readily over the 24 hour incubation period Glipizide under basic conditions demonstrated significantly less degradation overall with relatively small quantities of impurity 5 being generated over the 24hour period of incubation
All compounds of interest were identified with mass errors of between 0.8 and 4.1ppm.
Identification and visualisation of these compounds, and their associated fragments generated was automatically assigned using libraries within the processing method requiring no manual interpretation.

CONCLUSION

- **The Waters ACQUITY RDa was able to separate and successfully characterise Glipizide and its degradants without the need for HRMS expertise.**
- **Full scan with fragmentation function provided additional structural information for increased confidence in compound identification.**
- **These results were achieved without any manual calibration or setup of the ACQUITY RDa mass analyzer**
- **UNIFI as part of the waters_connect platform allows for a fully 21CFRPart11 compliant end-to-end workflow creation for routine analysis of forced degradation.**

References

1. Patolia VN. An Introduction To Forced Degradation Studies For Drug Substance & Drug Product. Pharmaceutical Online Jan 9, 2020
2. FDA Guidance for Industry, INDs for PhaseII and III Studies—Chemistry, Manufacturing, and Controls Information, Food and Drug Administration.

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