THE USE OF A TRIPLE DETECTION SYSTEM (UV, ELSD, MS) FOR PHARMACEUTICAL DEGRADATION STUDIES

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INTRODUCTION

In the pharmaceutical industry, there is a need to fully understand the stability of an active pharmaceutical ingredient (API) and characterize any impurities that may be formed, including those found in forced degradation studies. Reversed-phase liquid chromatography UV-based techniques are often used for these types of analyses. However, separating and detecting the related impurities and other components can be challenging. By combining a UV detector, an evaporative light scattering detector, and a mass spectrometer, it is possible to detect compounds with different chemical properties.

In the following study, we will conduct a forced degradation study which will also include mass balance studies using triple detection. Mass balance can be affected by the differences in response factors of the API and related impurities. We will perform two sets of experiments to determine the relative response factors (RRF) of the impurities. This process will include either: 1) using the ratio of the slopes of the API and related impurities or 2) comparing the UV peak area to the log of the ELSD peak area, the latter which has a response based on unit mass. Using the relative response factors we will then evaluate the mass balance of the acid hydrolysis of glimepiride. In addition, the QDa mass detector will provide added information for peak confirmation and peak purity.

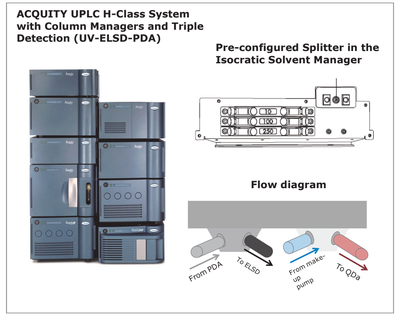

Figure 1. ACQUITY UPLC H-Class system with triple detection including ACQUITY PDA, ELSD and QDa detectors. The triple detection system includes an isocratic solvent manager (ISM) which provides make-up solvent to the QDa detector and houses the splitter required for the ELSD and the QDa. All of the flow is directed to the splitter from the PDA detector. A portion is then sent to the ELSD detector. Make-up solvent is introduced in the next port and the flow is then mixed as it flows into the QDa or mass detector. The composition and flow rate of the make-up solvent impact the split ratio to the ELSD and the QDa.

METHODS

Conditions

System: ACQUITY UPLC H-Class with Column Manager
Column: ACQUITY UPLC BEH C18, 1.7 µm, 2.1 x 50 mm
Mobile phase A: 0.1% (v/v) Formic acid in Water
Mobile phase B: 0.1% (v/v) Formic acid in Acetonitrile
Column Temperature: 30 C
Injection volume: 2 uL
Flow rate: 0.8 mL/min
Isocratic:60% A: 40%B
Gradient:

Time	%A	%B	%C	%D	Curve
Initial	95.0	5.0	0.0	0.0	Initial
5.00	5.0	95.0	0.0	0.0	6
6.50	5.0	95.0	0.0	0.0	6
6.51	95.0	5.0	0.0	0.0	6

ACQUITY PDA Detector
Wavelength range: 210-400 nm
Resolution: 3.6 nm
Selected wavelengths: 228 nm, 4.8 nm resolution
Time Constant: Normal
Sampling rate: 20 pts/s

ACQUITY Evaporative Light Scattering Detector (ELSD)
Gas: 25 psi
Neubilizer Mode: Cooling
Nebulizer Temperature: 55 °C
Gain: 350
Data rate: 10 pps

Isocratic Solvent Manager
Flow rate: 0.3 mL/min
Solvent: 0.1% (v/v) formic acid in methanol

ACQUITY QDa Detector (mass detector)
Mass range: 100-600
Cone voltage: 5V
Sampling Rate: 5 pps
Capillary Voltage: 1.4 kV

Sample Preparation:
Glimepiride and related compounds B and C were purchased from the USP. All standards were dissolved in 55:45 methanol:water and sonicated.

The drug substance glimepiride was obtained from an outside source. Acid hydrolysis was conducted at 40 °C for 0-7 days . The concentration of acid was 0.1M HCl in the degradation reaction.

Samples	Conditions
API Control	RT and 40°C for 1,3,5, 7 days
Acid control	RT and 40°C for 1,3,5, 7 days
Acid Hydrolysis	RT and 40°C for 1,3,5, 7 days

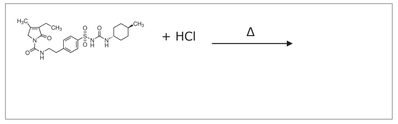

Figure 2. Forced degradation of glimepiride drug substance.

RESULTS AND DISCUSSION

Multi-detection of API and Related Compounds

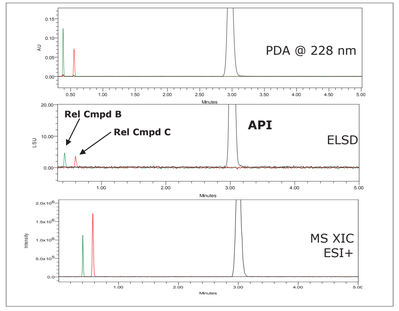

Figure 3. Separation of standards of active pharmaceutical ingredient (API), related compound B and related compound C under isocratic conditions. The overlay of standards at 250 µg/mL for the API and 10 µg/mL for related compounds B and C shows the differences in relative response among the detectors. The UV and ELSD give similar relative response for the three compounds. In the mass detector related compound C has a greater peak area than related compound B.

Determination of Relative Response Factors Using Slope of Calibration Curves in UV

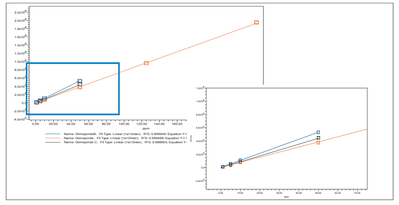

Figure 4. Overlay of UV linearity curves at 228nm of glimepiride and related impurities B and C. The API linearity curve covers the range of 1-250 µg/mL for the API and 1-50 µg/mL for related compounds B and C. All R^2 values were >0.998.

$$RRF = \frac{[Slope\ of\ Impurity]}{[Slope\ of\ API]}$$

Wavelength	Separation Conditions	RRF Rel Compound B	RRF Rel Compound C
228	Isocratic	**1.36**	1.10

Table 1. Relative Response Factors for Related compound B and C using the ratio of the slope of the API/slope of the impurity. The value for related compound B is outside of 0.8-1.2 range and, therefore, should be applied, as specified by the USP Chapter <621>.[1]

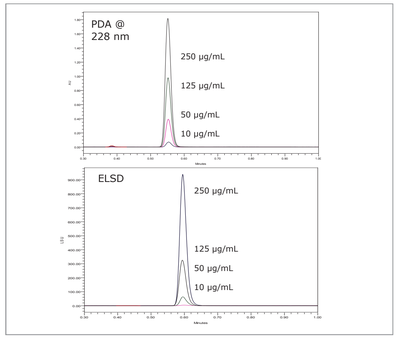

Figure 5. Overlay of glimepiride related compound C standards (10-250 µg/mL) in PDA and ELSD. The UV detector produces a linear response for standards. Evaluating the peak areas in the ELSD, a non-linear or logarithmic response is observed. For example, at 10 µg/mL the response in the ELSD (pink trace) is significantly lower than that observed in the PDA.

Determination of Relative Response Factors Using Ratio of UV to ELSD (log) Peak Area

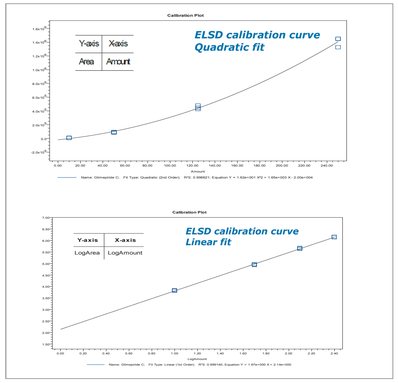

Figure 6. ELSD calibration curves for glimepiride related compound C. The ELS detector has a quadratic fit to the calibration curve (top) for peak area vs. the amount. If the values are converted to the logarithmic functions (inset), the calibration curve fit is linear (bottom). The R^2 value for this curve is 0.999140.

$$RRF = \frac{UV\ Area_{Impurity}}{Conc\ Detector\ response_{\ impurity}} \Big/ \frac{UV\ Area_{API}}{Conc\ Detector\ response_{\ API}}$$

$$RRF = \frac{UV\ Area_{Impurity}}{ELSD\ \log(Area_{\ impurity})} \Big/ \frac{UV\ Area_{API}}{ELSD\ Area_{\ API}}$$

Standard	RRF Rel Compound B	RRF Rel Compound C
50	**1.25**	1.10
125	**1.24**	1.09

Table 2. Relative Response Factors for related compound B and C using the ratio of the UV peak area to the log of the ELSD peak area. RRF can be calculated using the response of the UV detector to a mass concentration dependent detector.[2] This assumes a linear relationship for both detectors. To convert the ELSD calibration response to a linear function, the log of both x and y values can be used. Thereby, using the log of the ESLD peak area, we can calculate RRF factors for both impurities. These values have good correlation with those obtained using the slopes of the calibration curves in the UV.

Mass Balance for Forced Degradation Studies

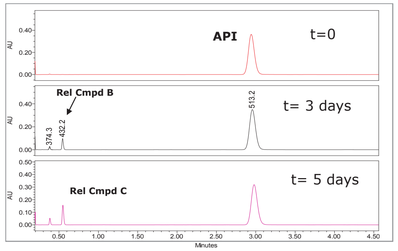

Figure 7. UV chromatograms of forced degradation of glimepiride drug substance with base mass labels. The drug substance was exposed to acidic hydrolysis conditions at 40 C over a period of days. Over the course of the study the two impurity peaks (related compound C and B) increased in peak area.

n = 3	Reference	T=0	1 day	3 days	5 days	7 days
Amount	238.4	236.1	239.0	242.7	239.1	244.1
% Recovery		99.0	100.2	101.8	100.3	102.4

Table 3. Mass balance determinations for forced degradation of glimepiride. The calculations were performed using RRF determined with the ELSD method. The RRF were entered into Empower 3 FR 2 for corrected values of the related impurities. All mass balance values were within 2%.

Ensuring Peak Purity using Mass and UV detection

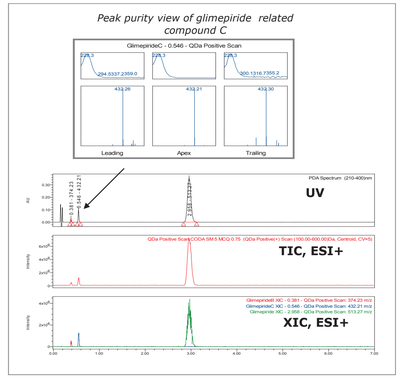

Figure 8. Mass analysis window in Empower 3 FR2 of forced degradation at 3 days. In forced degradation studies, mass imbalance can be caused by co-elutions of the API and any impurities. To evaluate peak purity, mass detection can provide valuable information. In this view, the UV, Total Ion Chromatogram (TIC) and Extracted Ion Chromatograms (XIC) are shown in a stacked plot. For each integrated peak in the UV chromatogram the leading, apex and trailing portion of the peak can be viewed (top portion of figure). Using the mass and UV detectors the peak purity can be evaluated to ensure no co-elutions.

CONCLUSIONS

- **Triple detection system in combination with Empower 3 FR2 provides various tools to assist in mass balance, including:**
  - **Determination of relative response factors by using the ratio of UV peak and the log of ELSD peak responses**
  - **The ability to input relative response values into Empower 3 FR2 to determine corrected area values for impurities for mass balance determinations**
  - **Using orthogonal detection (UV and mass) to confirm peak purity and to detect the presence of co-elutions that could impact mass balance.**

References

1. Chapter <621> CHROMATOGRAPHY United States Pharmacopeia and National Formulary (USP 37-NF 32 S1) Baltimore, MD: United Book Press, Inc.; 2014. p. 6376-85.
2. Mark AN, Andreas K, Patrick JJ. Role of Mass Balance in pharmaceutical stress testing. Pharmaceutical Stress Testing: CRC Press; 2011. p. 233-53.
3. Bansal G, Singh M, Jindal KC, Singh S. LC–UV–PDA and LC–MS studies to characterize degradation products of glimepiride. Journal of pharmaceutical and biomedical analysis. 2008; 48:788-95.

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