USE OF A TRIPLE DETECTION (UV-ELSD-MS) SYSTEM FOR MASS BALANCE IN FORCED DEGRADATION OF PHARMACEUTICALS

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INTRODUCTION

Forced degradation studies are typically performed using HPLC and UV detectors to understand the degradation pathway of pharmaceuticals and to insure all impurities are accounted. In these studies, performing mass balance or the conservation of mass is crucial. Multiple orthogonal detectors based on different principles can be used to measure or identify compounds with different chemical or physical properties. We will evaluate mass balance using a triple detection system consisting of a PDA, ELSD and a mass detector. Relative response ratios will then be used to perform mass balance. The degradation path way will then be confirmed using of a mass detector, specifically through the identification of impurities and their by-products.

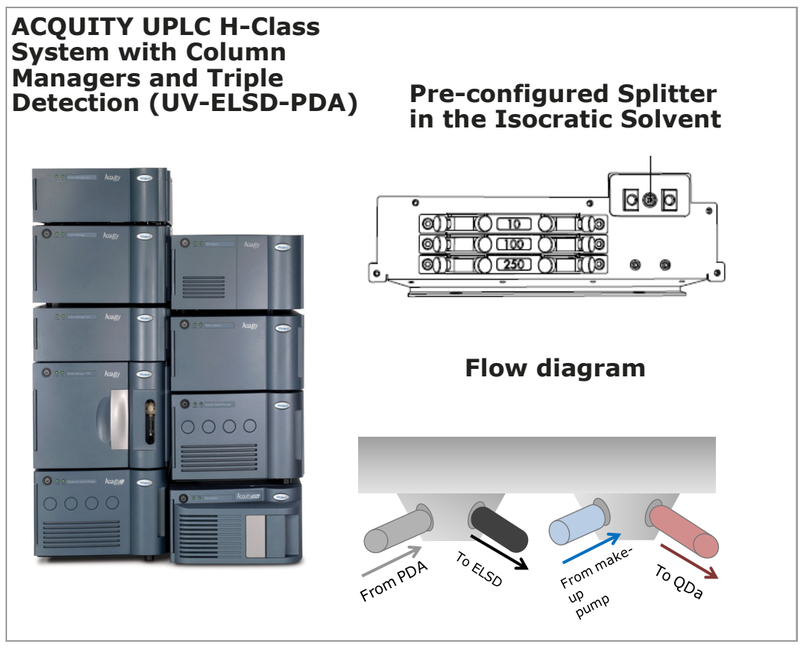
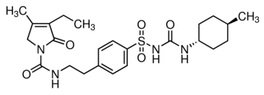


Figure 1. ACQUITY UPLC H-Class system with triple detection including ACQUITY PDA, ELSD and QDa detectors. The triple detection system includes an isocratic solvent manager (ISM) which provides make-up solvent to the QDa detector and houses the splitter required for the ELSD and the QDa. After the PDA detector, the flow is split to the ELSD detector and QDa. 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



glimepiride

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 ELSD Detector
Gas: 25 psi
Data rate: 10 pps
Nebulizer Mode: Cooling
Nebulizer Temperature: 55 °C

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

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.

RESULTS AND DISCUSSION

Multi-detection of API and Related Compounds

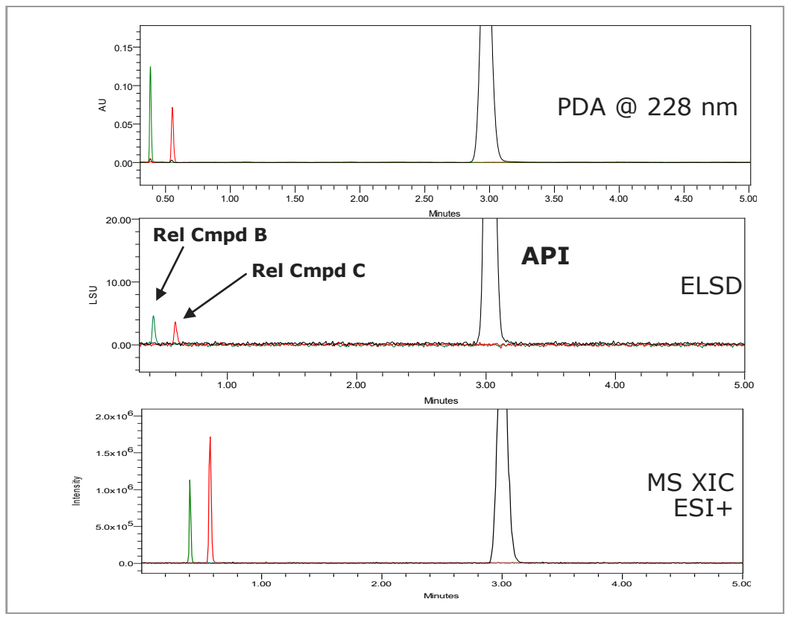


Figure 2. Separation of standards of active pharmaceutical ingredient (API), related compound B and related compound C under isocratic conditions. The overlay of standards at 250 µg/mL for the API and 10 µg/mL for related compounds B and C shows the differences in relative response among the detectors. 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

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

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]

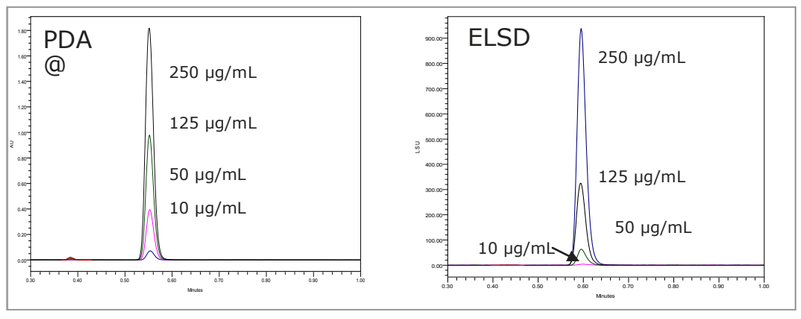


Figure 3. Overlay of glimepiride related compound C standards (10-250 µg/mL) in PDA and ELSD. The UV detector produces a linear response for standards. Evaluating the peak areas in the ELSD, a non-linear or logarithmic response is observed. For example, at 10 µg/mL the response in the ELSD (pink trace) is significantly lower than that observed in the PDA.

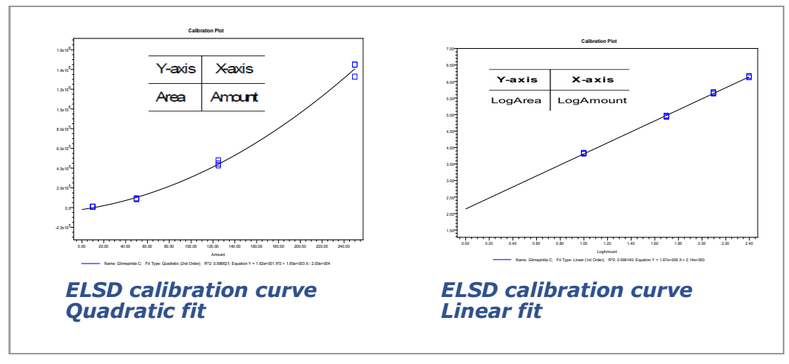


Figure 4. ELSD calibration curves for glimepiride related compound C. The ELS detector has a quadratic fit to the calibration curve (left) for peak area vs. the amount. If the values are converted to the logarithmic functions (inset), the calibration curve fit is linear (right). The R^2 value for this curve is 0.999140.

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

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

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

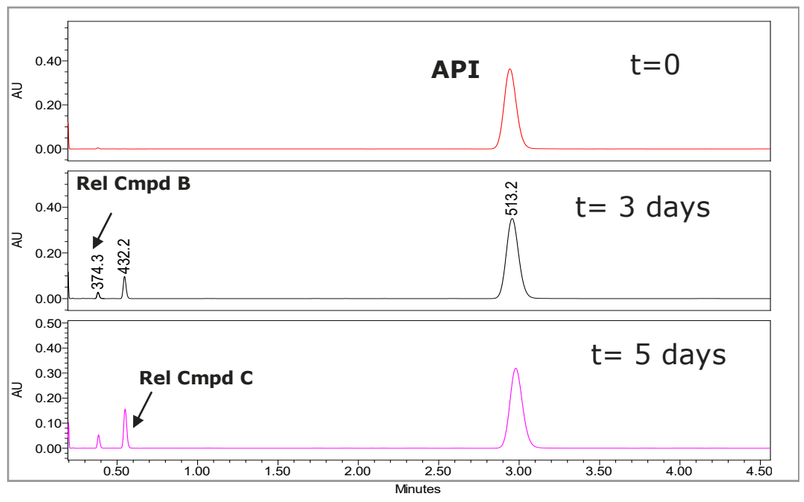


Figure 5. UV chromatograms of forced degradation of glimepiride drug substance with base mass labels. The drug substance was exposed to acidic hydrolysis conditions at 40 C over a period of days. Over the course of the study the two impurity peaks (related compound C and B) increased in peak area.

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%.

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 to determine corrected area values for impurities for mass balance determinations**

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.

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