

STRATEGIES TO EVALUATE AND MONITOR FORCED DEGRADATION STUDIES USING A DUAL DETECTION (UV-MS) SYSTEM

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INTRODUCTION

Forced degradation studies are typically performed to understand the degradation pathway of pharmaceuticals. Given the range of impurities and their chemical and physical properties, a single detection technique may not be adequate to accurately measure all of the degradants.

To address the challenges of measuring and quantifying degradants, a dual detection system consisting of a photodiode array (PDA) and a mass detector (MS) will be used. Orthogonal detection will be used to illustrate the impact of co-elutions on mass balance determinations. The addition of MS information, whether for non-chromophoric species or co-elutions, will allow for a more complete mass balance evaluation and more comprehensive understanding of the degradation pathway.

METHODS

System: ACQUITY UPLC H-Class with PDA and QDa Detector

Method Development Conditions:

Column: ACQUITY UPLC BEH C18 1.7µm, 2.1 x 50 mm
Mobile phase (prepared using AutoBlend): A: 125 mM Formic acid, B: 125 mM Ammonium hydroxide C: Water; D: Acetonitrile
Screening gradient: 5-90% Acetonitrile in 5 min (10% A or B, constant)
Optimized gradient: 30-55% acetonitrile + 12.5 mM NH4OH in 5 min, 55-80% in 2 min
Flow rate: 0.6 mL/min
Wavelength: 254 nm

MS Settings:

Mode: Electrospray (+)
Mass range: 50-500 m/z

Sample Preparation:

Loratadine drug substance (Toronto Chemical Research) stock solution was degraded at 0.2mg/mL with H₂O₂ (1.2%) (Sigma Aldrich). The sample was tested after 3 days at room temperature.

MS Quantification Conditions:

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
Flow rate: 0.8 mL/min
Isocratic: 60% A: 40%B
Wavelengths: 228 nm, 4.8 nm resolution
Sampling rate: 20 pts/s

MS Settings:

Mode: Electrospray (+)
Mass range: 100-600 m/z
Single Ion Recording channels (SIR): 114.1m/z

Sample Preparation:

Glimepiride drug substance glimepiride was obtained from an outside source. Oxidation by AIBN was conducted at room temperature for 0-7 days.

Chromatography Data System: Empower 3 FR 3

Method Development of Forced Degradation Studies

Method development typically employs a fast LC-UV screening method. This approach may include numerous columns, mobile phases and conditions. Assessing separation quality by UV alone can provide challenges in determining co-elutions and peak tracking. By incorporating MS, orthogonal detection can be employed for added information.

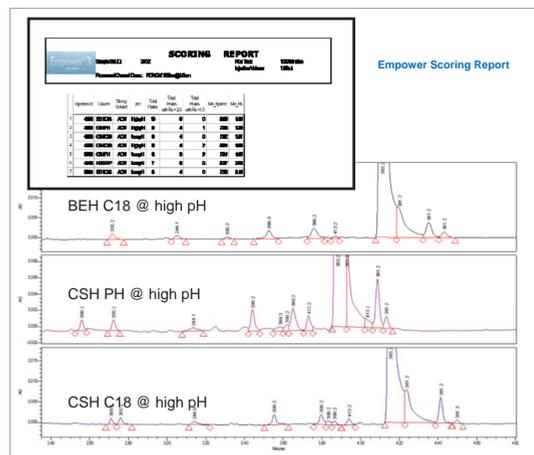


Figure 1. Screening of oxidized loratadine drug substance across multiple columns. Analyses were processed and evaluated in Empower using a scoring report, based on pre-defined criteria (greatest number of peaks, etc.). BEH C₁₈ column at high pH was selected for method optimization. Base mass annotated above UV peaks are used to assist in peak tracking.

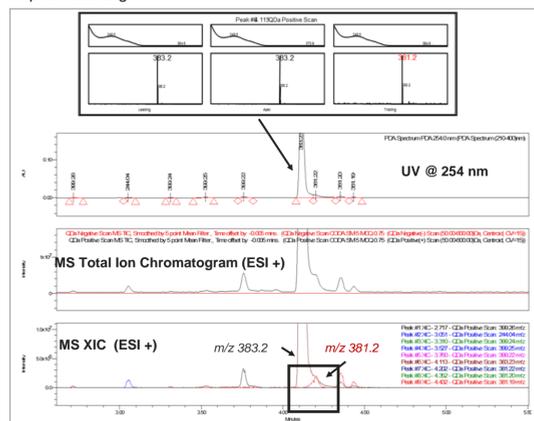


Figure 2. Mass analysis window view in Empower 3 CDS of loratadine oxidized sample (t = 3d). Screening method (5-90% D in 5 min) was used, resulting in co-elutions (box at top). Evaluation of UV and MS Total Ion chromatograms show co-elution of the API and impurity (black box). This was confirmed by eXtracted Ion Chromatogram (XIC) (bottom box).

RESULTS AND DISCUSSION

Screening methods can produce co-elutions, which impact identification and quantification of the formed degradation products. MS can be added to provide an orthogonal mode of detection to assist in peak tracking and provided added confidence in the final separation. Peak purity can be confirmed by both spectroscopic means, providing added assurance that no co-elutions are present.

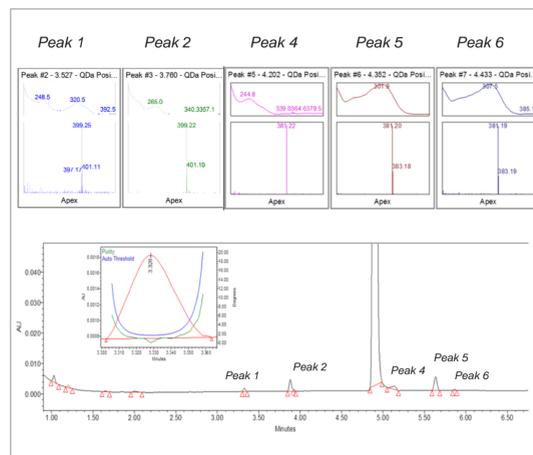


Figure 3. Optimized method for loratadine forced degradation sample with separation of previously co-eluting pair. UV peak purity performed using Max Plot chromatogram, all peaks met purity threshold (UV peak purity shown in inset). Peak purity verified by MS. Based on MS information, standards were purchased to confirm identity of peak 1 (n-oxide) and 2 (epoxide).

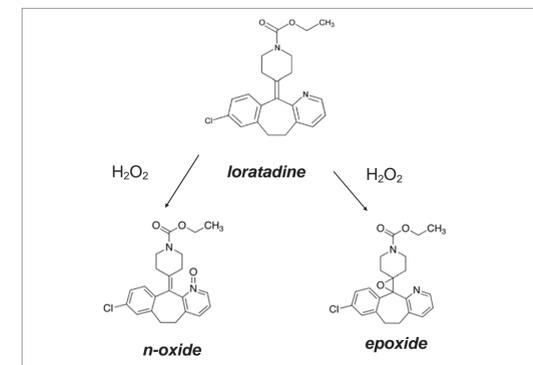


Figure 4. Degradation pathway of loratadine oxidation resulting in formation of oxide impurities. Isobaric impurities were confirmed by standards and MS.

MS for Quantification of Non-Chromophoric Species

When conducting forced degradation studies, impurities with a variety of chemical and physical properties may be formed. These include compounds with no or low chromophores, which may not be measured by UV detection. To assess or measure these components, mass detection can be added as an orthogonal detection technique.

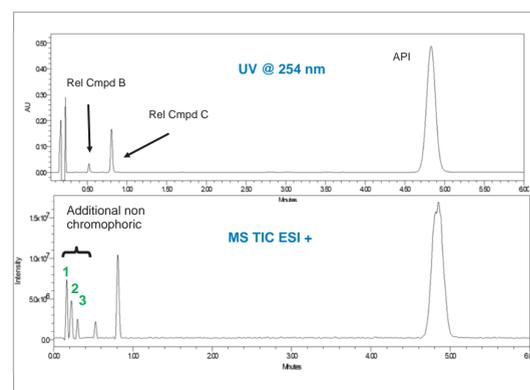


Figure 5. Forced degradation (oxidation by AIBN) of glimepiride at 5 days produced related compound B and related compound C as shown in UV chromatogram. Analysis by MS indicates presence of three additional peaks.

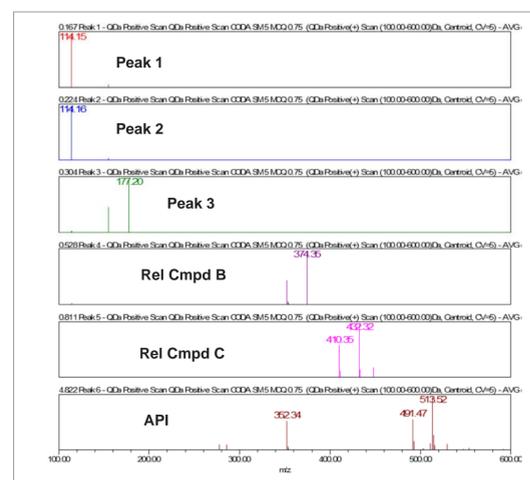


Figure 6. Mass spectral information for peaks in MS TIC ESI+ of glimepiride oxidation by AIBN. Spectral information of peaks 1 and 2 indicate isobaric species. Peak 3 is from AIBN reagent, and confirmed in blank. Rel compounds B and C, and API were confirmed by mass spectral information (Na⁺ adducts were present).

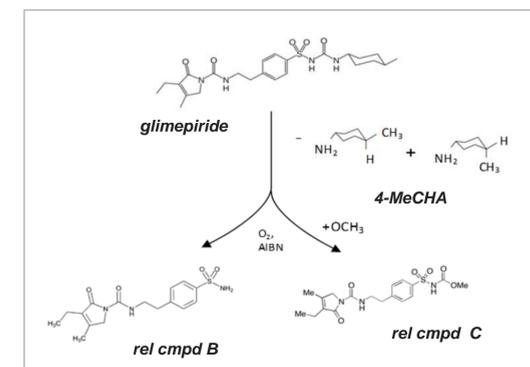


Figure 7. Oxidative degradation pathway of glimepiride to form related compound B and C. Both reactions produce 4-methylcoamine (4-MeCHA) as a non-chromophoric by-product.

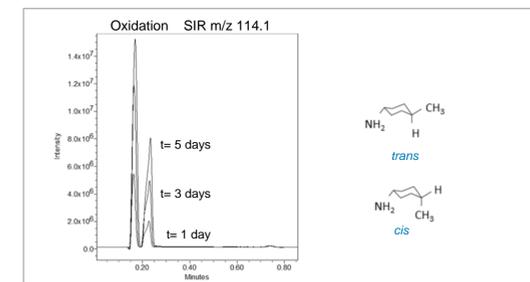


Figure 8. Monitoring 4-MeCHA byproduct in oxidative degradation at 1, 3, 5 days. Single Ion Recording channel (SIR) of m/z 114.1 (for 4-MeCHA) shows presence of two isomers (cis and trans). Area of each isomer increases with oxidation time.

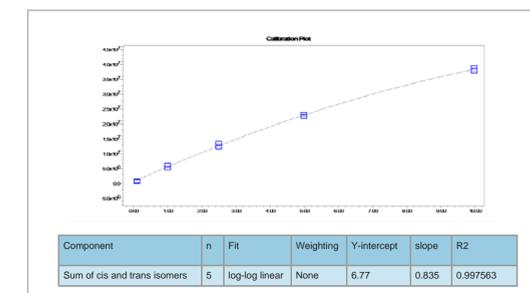


Figure 9. MS calibration curve of 4-MeCHA standard, sum of cis and trans isomers were used. Calibration curve had a quadratic fit from 0.1 to 10 µg/mL.

Time (days)	4-MeCHA (µg/mL) (% Area)	Mass Balance (%)	Mass Balance (%) + 4-MeCHA
1	1.48	100.70	101.30
3	4.02	97.00	98.60
5	6.70	96.92	99.60
7	13.69	97.21	101.60

Figure 10. Quantification of 4-MeCHA and its impact on mass balance. As described previously the non-chromophoric by-product, 4-MeCHA, was observed by MS. Quantification of the by-product was performed using purchased standards (Figure 9). At longer time periods (7 days), increased amounts of the by-product were formed resulting increase in mass balance (based on weight).³

CONCLUSIONS

- Mass detection aides in method development by providing an additional tool to perform peak tracking and assess co-elutions
- UV-MS detection can be used to assess peak purity by spectroscopic and mass spectral means, increasing assurance of no co-elutions
- Mass spectroscopy provides a tool for detecting non-chromophoric degradants and by-products in forced degradation reactions, providing both greater understanding of the degradation pathway and a more complete mass balance

1. Chapter <621> CHROMATOGRAPHY United States Pharmacopeia and National Formulary (USP 37-NF 32 S1) Baltimore, MD: United Book Press, Inc., 2014, p. 6370-65.
2. 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.
3. Hong P, Phoebel AD, Jones MD. Study of relative response factors and mass balance in forced degradation studies with liquid chromatography/photo-diode array detector/evaporative light scattering detector/mass spectrometry system. Journal of Chromatography A. 2017; 1512:61-76.