FRACTION COLLECTION FOR ISOLATING IMPURITIES IN FORCED DEGRADATION STUDIES

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INTRODUCTION

Forced degradation studies are typically performed to understand the degradation pathway of pharmaceuticals. One of the specific challenges includes determining the response factor of impurities relative to the active pharmaceutical ingredient (API). Incorrectly identifying the relative response factors (RRFs) could lead to over or under quantification of the impurity, which can in turn lead to mass imbalance. In this presentation the impurity will be collected from the forced degradation analysis by small scale fraction collection and subsequently used to determine RRFs. The RRFs will be also evaluated by established methodologies, specifically comparison of the calibration curve of both the API and impurity standard.

Figure 1. Waters Fraction Manager - Analytical (WFMA)

METHODS

Conditions for forced degradation studies:

System: ACQUITY UPLC H-Class with PDA and QDa Detector
Column: ACQUITY UPLC BEH C18 1.7µm, 2.1 x 50 mm
Column Temperature: 30 °C
Injection volume: 4 µL (or 10 µL for scale up experiment)
Mobile phase (prepared using AutoBlend):
B– 125 mM Ammonium hydroxide C– Water; D– Acetonitrile
Flow rate: 0.6 mL/min
Wavelength: 254 nm

Time	%A	%B	%C	%D
0.00	0	10	60	30
5.00	0	10	35	55
7.00	0	10	10	80

MS Settings:
Mode: Electrospray (+)
Mass range: 50-500 m/z
Capillary Voltage: 1.5 kV
Cone Voltage:15 V
Single Ion Recording channels (SIR): 383.3, 399.3
Make up pump (ISM): 0.3 mL/min, 0.1% formic acid in methanol

Conditions for collection scale– up:
System: ACQUITY UPLC H-Class with PDA Detector and WFMA
Column: XBridge BEH C18 2.5 µm, 3.0 x 75 mm
Column Temperature: 30 °C
Injection volume: 30.6 µL
Mobile phase (same as described for forced degradation)
Flow rate: 0.833 mL/min

Sample preparation: Oxidation of loratadine drug substance

Loratadine and related impurities (n-oxide and epoxide) were purchased from the Toronto Research Chemicals. All standards were dissolved in 1:1 methanol:water and sonicated. The loratadine drug substance was exposed to oxidative conditions (3% H_2O_2) at 70 ˚C for up to 90 minutes.

Sample preparation for collected fractions

Approximately 70 injections (over a period of 23 hours) of the forced degradation sample were run on a 3.0 x 75 mm column. Both impurity 1 (n-oxide) and impurity 2 (epoxide) were collected and pooled (per peak). The samples were then dried down to remove the organic portion, frozen and then lyophilized. The dried samples were reconstituted with 500 µL of methanol.

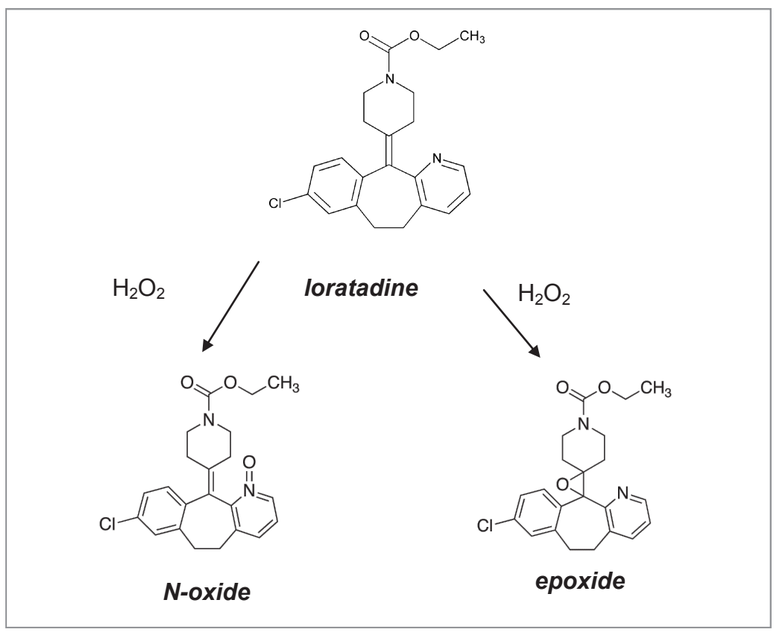

Figure 2. Pathway for oxidative degradation of loratadine.

RESULTS AND DISCUSSION

FORCED DEGRADATION ANALYSIS AND FRACTION COLLECTION

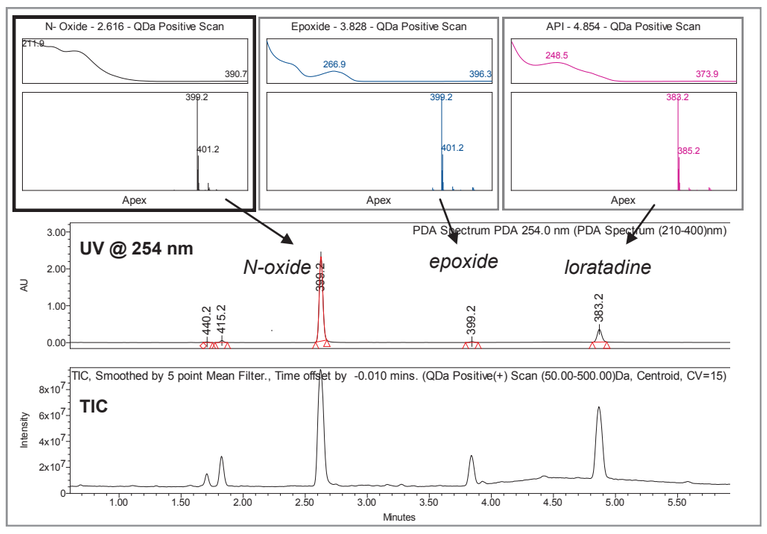

Figure 3. Stacked view of UV and mass total ion (TIC) chromatogram of forced degradation of loratadine drug substance with base mass labels. The drug substance was exposed to oxidative conditions at 70 °C for 90 min. A major impurity peak (76% area) was observed at 2.616 min, while a minor peak (0.88% area) was observed at 3.828 min. Both peaks had a base mass of 399.2 suggesting oxidation of the API at a single site.

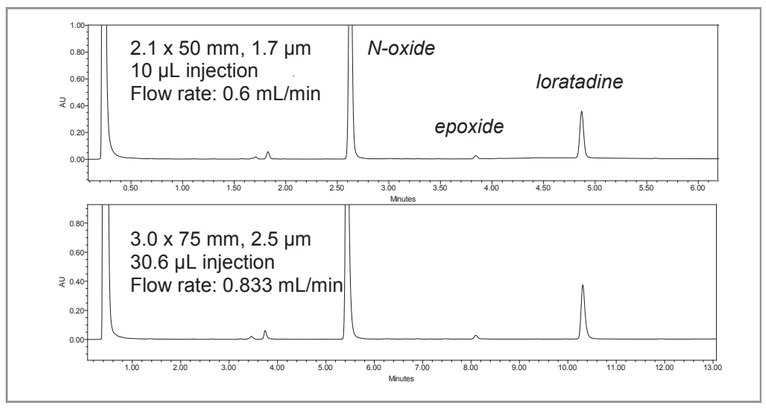

Figure 4 Chromatograms on both a 2.1 x 50mm and 3.0 x 75 mm column, the latter of which was used for fraction collection. Scaling to the 3.0 mm ID column allowed a larger injection volume per injection. Multiple injections were pooled to collect adequate amount of sample.

*Figure 5. Software used to set up fraction collection. **Collection Event Table** (upper right) allows events to be imported from a processed chromatogram (or result) using **Fill From Chromatogram** (upper left). In this window a peaks table is populated based on selected result. User has the option to select which peaks to import into collection table. **Simulation** option allows analyst to visualize peak collection as compared to a chromatogram (lower views).*

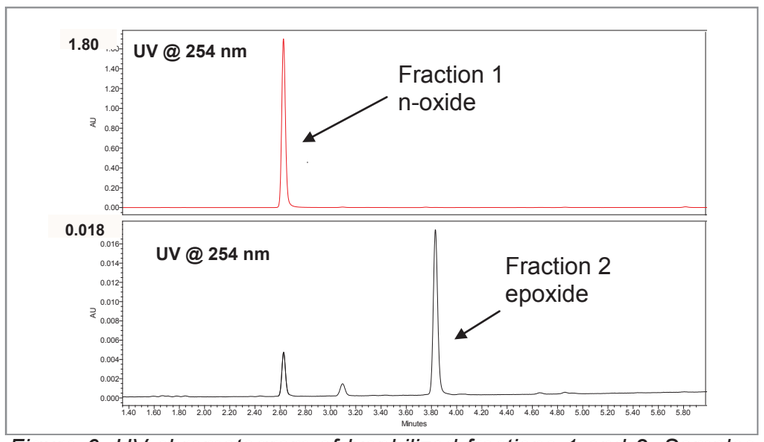

Figure 6. UV chromatogram of lyophilized fractions 1 and 2. Samples were reconstituted in 500 µL of methanol. Quantification of the fractions showed collection of 188 µg of N-oxide and 12 µg of epoxide. Presence of additional peaks in fraction 2 may be attributed to degradation or conversion of the epoxide impurity during lyophilization.

RELATIVE RESPONSE FACTORS

To evaluate the relative response factors (RRF) for both related impurities, the standard curves were used. This approach uses the ratio of the slopes of the impurity and API calibration curves.[1] This requires a known amount of sample. For the lyophilized samples, weighing the small amounts of the impurities was challenging. Therefore, the amount of each impurity collected was calculated using calibration curves of the readily available standards. This also allowed for confirmation of the n-oxide and epoxide impurities.

Compound	Range (µg/mL)	R^2	Slope	RRF
Loratadine	1-500	0.996	16332632	1.0
Standards				
N-Oxide	1-500	0.998	16150615	1.1
Epoxide	1-500	1.000	3794973	0.2
Collected Fractions				
N-Oxide	1– 377	0.998	19771229	1.2
Epoxide	0.8-23	1.000	5021606	0.3

Table 1. Calibration curves and relative response factors (RRF) for loratadine and degradation products. Upper limit of calibration curve range for collected samples was limited by the amount of samples. RRF values for both sets of samples were comparable and within acceptable range.

MASS BALANCE

To assess the impact of RRF, oxidative degradation of loratadine drug substance was performed at 70 °C. The samples were analyzed after 30, 60 and 90 minutes.

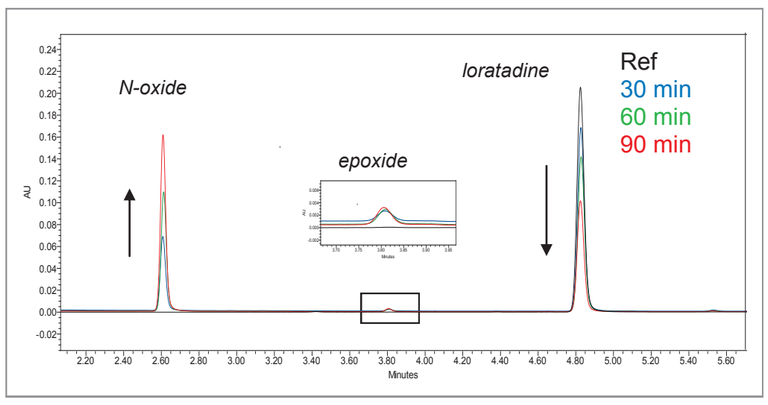

Figure 7. Oxidation of loratadine at various time points over 90 minutes. With increasing time, the drug substance peak area decreased and was accompanied by a significant increase of n–oxide (49% at 90 minutes). The epoxide impurity increased minimally from 0.6 to 0.9 % (see inset).

Mass balance calculations were performed using the peak area. Peak areas for n-oxide and epoxide impurities were adjusted using the RRF values (*). In general, RRF > 1.0 result in a decrease in peak area, while RRF < 1.0 result in an increase in peak area. Given the relative amount of n-oxide formed in the degradation and its RRF of >1.0, a decrease in mass balance was observed using the RRF values.

Time	Total Area (µV*sec)	Apparent Mass Balance	Total Adjusted Area *(µV*sec)	Corrected Mass Balance*
Reference	534488	100.0	N/A	N/A
30 min	596388	112	582878	109
60 min	604194	113	581550	109
90 min	629781	118	592461	111

Table 2. Comparison of mass balance calculations without and with (*) RRF corrections. Use of RRF resulted in a decrease in mass balance with values closer to 100% than the apparent mass balance.

CONCLUSION

- **Fraction collection of stressed drug substance can be performed on an analytical scale for multiple peaks in a single analysis**
- **Collection and pooling of multiple injections can be used to acquire micrograms of material**
- **Collected samples can be used to assess relative response factors by comparison of the standard curves to that of the drug substance.**

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.

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