ISOLATION OF A DEGRADATION PRODUCT OF RANITIDINE HYDROCHLORIDE USING THE WATERS ANALYTICAL SCALE PURIFICATION SYSTEM

Authors: Margaret Maziarz, Ronan P. Cleary, Darcy Shave, Stephanie N. Harden, Paul D. Rainville, Jinxia Xia
Affiliations: Waters Corporation, 34 Maple Street, Milford MA 01757

INTRODUCTION

Isolation of unknown components for structural characterization and identification is an important aspect of pharmaceutical development. The presence of unidentified components, including impurities and degradation products, may compromise the quality and safety of pharmaceutical products, posing health risks[1,2].

This work demonstrates the isolation of a degradation product of ranitidine hydrochloride (HCl) drug using the Waters Analytical Scale Purification System. The purification system configured with PDA mass detectors enabled UV- and mass-directed isolation of the degradation product formed during a forced degradation study under basic conditions. The preparative analysis was run on a 10 mm ID x 50 mm column with 3.5 µm particle size. The loading capacity was evaluated to increase the yield of the degradation product. The effectiveness of the isolation process was assessed by verifying chromatographic and spectral purity of the collected degradation product.

METHODS

Ranitidine drug substance (DS) and tablet samples

- Ranitidine DS samples were prepared in water at 1 mg/mL.
- Crushed tablets were dissolved in water at 1 mg/mL of ranitidine. Samples were sonicated for 10 minutes, centrifuged for 15 minutes, and filtered using 0.2 µm PTFE syringe filters.
- Control test samples were prepared by diluting 1 mg/mL sample solution with water to the working concentration of 0.1 mg/mL.

Forced degradation study

Ranitidine DS and tablet sample solutions at 1 mg/mL were forced degraded with 0.5 M hydrochloric acid and 0.5 M sodium hydroxide (NaOH) at room temperature for 3, 6, and 24 hours. At each interval, samples were neutralized with equal volumes of equivalent concentration of base and acid. All solutions were diluted with water to the working concentration of 0.1 mg/mL.

Parameter	Analytical method	Preparative method
Systems	• Arc™ Premier System with Quaternary Solvent Manager (QSM-R) and Flow-Through Needle (FTN-R) • 2998 PDA Detector • ACQUITY™ QDa II™ Detector	Waters Analytical Scale Purification System composed of • Two Arc HPLC Quaternary Solvent Manager (QSM-R) pumps • 3767 Sample Manager • Isocratic Solvent Manager (ISM) • CM-30S Column Manager • 2998 PDA Detector and ACQUITY QDa II Detector
Column	XBridge™ Premier BEH™ C_{18} Column, 4.6 mm ID x 50 mm, 3.5 µm (p/n: 186010658)	XBridge Premier Peptide BEH C_{18}, 130Å, OBD™ Prep Column, 3.5 µm, 10 mm ID x 50 mm (p/n: 186011325)
L/dp	14,286	14,286
Gradient	Time (min) / FR (mL/min) / %A / %B / Curve Initial 1.50 95.0 5.0 6 4.00 1.50 60.0 40.0 6 4.50 1.50 5.0 95.0 6 5.00 1.50 5.0 95.0 6 5.10 1.50 95.0 5.0 6 7.00 1.50 95.0 5.0 6	Time (min) / Total FR (mL/min) / %A / %B / Curve Initial 4.00 95.0 5.0 Initial 0.50 8.00 95.0 5.0 6 3.50 8.00 60.0 40.0 6 4.00 8.00 5.0 95.0 6 4.50 8.00 5.0 95.0 6 4.60 8.00 95.0 5.0 6 5.50 8.00 95.0 5.0 6 5.90 4.00 95.0 5.0 1 6.00 4.00 95.0 5.0 6
Inj. volume	15.0 µL	71.0 µL
Chromatography data software (CDS)	Empower™ CDS 3.8.0	MassLynx™ Software version 4.2 SCN1049 FractionLynx™ Application Manager

Table 1. Method conditions.
Mobile phase: water with 0.1% formic acid (A) and methanol with 0.1% formic acid (B). Column temperature: 30°C, UV detection: 210 – 400 nm, derived at 235 nm, FR: flow rate.

RESULTS

The forced degradation study resulted in the formation of a degradation product under basic condition (Figure 1).

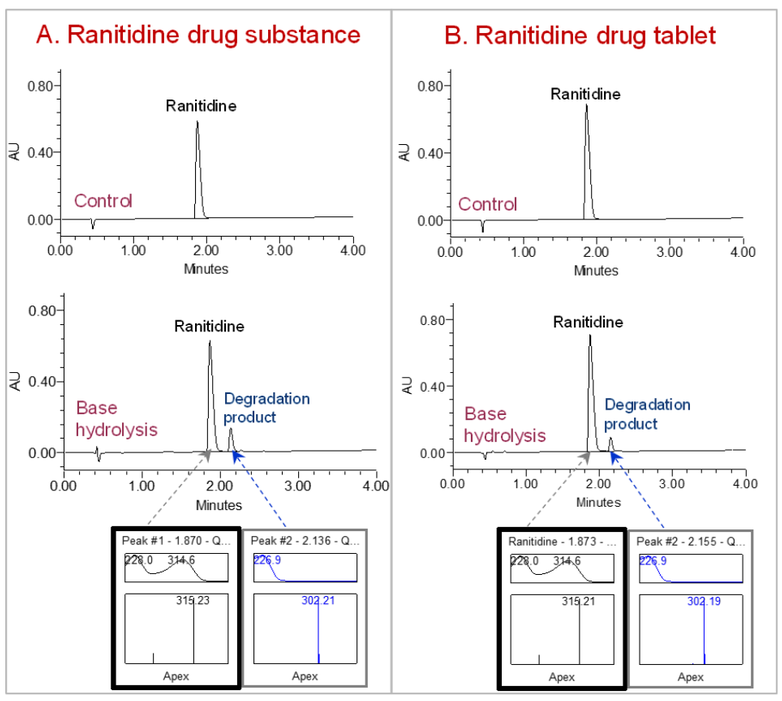

Figure 1. Analysis of ranitidine drug substance (A) and ranitidine drug tablet formulation (B) sample solutions using analytical method: Arc Premier System with XBridge Premier BEH C_{18} Column, 4.6 x 50 mm, 3.5 µm . Hydrolysis with base generated a degradation product with m/z 302.

To isolate the degradation product, the analytical method was scaled-up to preparative conditions, followed by fraction collection.

Scale-up and preparative analysis

The Waters Preparatory OBD Columns Calculator was used to aid the scale up process from analytical to preparative conditions[4].

The analytical method was scaled to preparative conditions using a column with the same chemistry and L/dp ratio (ratio of column length to the particle size).

The preparative analysis was run on a 10 mm ID x 50 mm column with 3.5 µm particle size (Figure 2). To accommodate the use of 3.5 µm particle size column, higher pressure pumps were utilized. The flow rate was adjusted and split equally between the two pumps. The gradient was modified to assure resolution between the peaks to ensure collection of high purity material.

Purity of the collected fractions

To assess the effectiveness of the isolation process, chromatographic and spectral purity of the collected degradation product were verified by injecting MS-triggered fractions on the analytical Arc Premier System controlled by Empower Software (Figure 3).

The chromatographic purity or percentage (%) area calculated by comparing area of the degradation product to the total area in the chromatographic injection was found to be 99.7%.

Spectral purity, verified using both the UV spectral data and mass spectral data, demonstrated that the degradation product was spectrally homogenous and not subject to coelution with any other components from the sample.

Loading study

Achieving high load capacity is essential in increasing the yield of an isolated material, producing larger amounts for further testing and characterization. While increasing the injection volume generally produces higher yield of the collected material, sufficient chromatographic resolution between the peaks with no interference must be obtained to produce high purity material.

In this work, the loading capacity was evaluated by collecting MS-triggered fractions of the degradation product peak using different injection volumes ranging from 100 to 600 µL (Figure 4). The collected fractions were then injected on the analytical Arc Premier System to assess chromatographic purity of the degradation product. The chromatographic purity ranged from 97.5% to 96.3%.

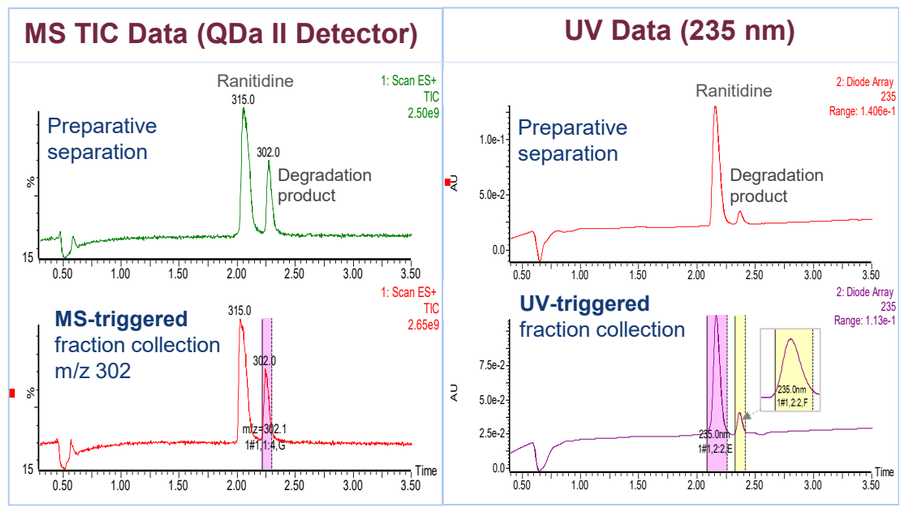

Figure 2. Preparative analysis and isolation of the degradation product using the Waters Analytical Scale Purification System integrated with PDA Detector and an ACQUITY QDa II Detector. Fraction collection through MS– and UV-triggers.

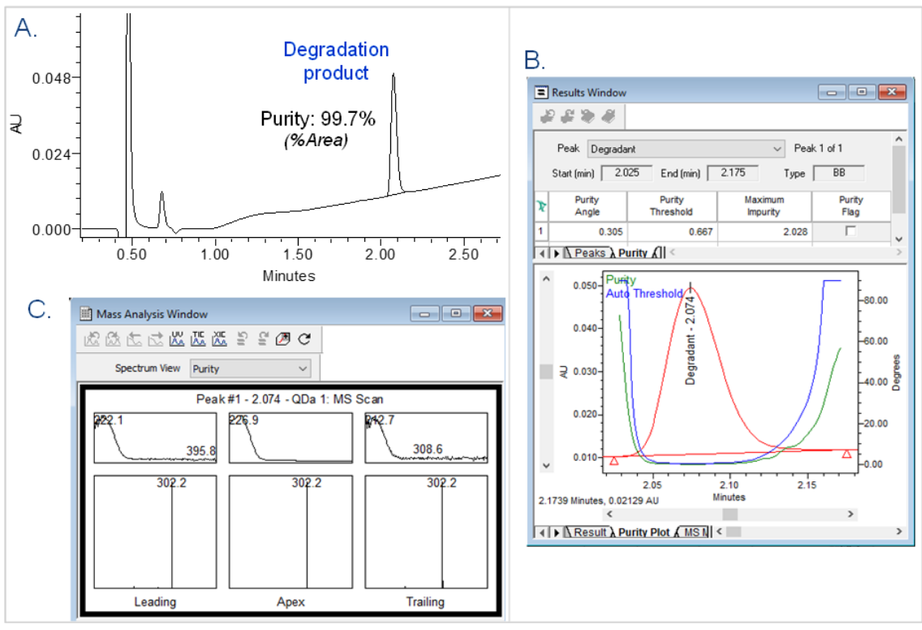

Figure 3. Purity determination of a fraction collected by MS-trigger. Analysis run on the analytical Arc Premier System, UV at 235 nm. Chromatographic separation (A), UV Peak Purity Plot (B), and Mass Analysis Window with purity spectrum (C).

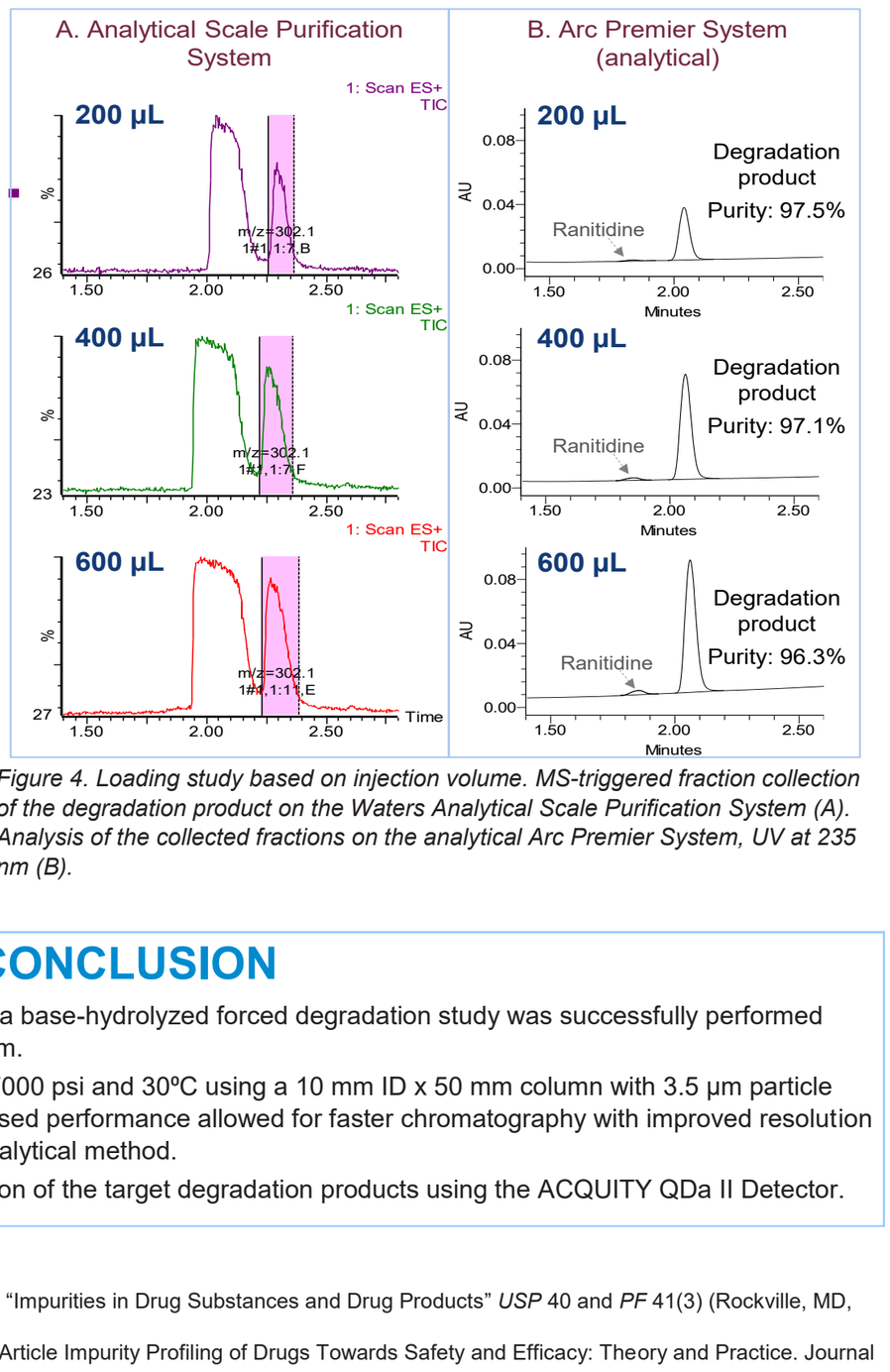

Figure 4. Loading study based on injection volume. MS-triggered fraction collection of the degradation product on the Waters Analytical Scale Purification System (A). Analysis of the collected fractions on the analytical Arc Premier System, UV at 235 nm (B).

CONCLUSION

- Isolation of the degradation product formed through a base-hydrolyzed forced degradation study was successfully performed using the Waters Analytical Scale Purification System.
- The preparative separation was done at 8 mL/min, 7000 psi and 30ºC using a 10 mm ID x 50 mm column with 3.5 µm particle size, to match the analytical separation. This increased performance allowed for faster chromatography with improved resolution and sensitivity. It also simplified scaleup from the analytical method.
- MS-directed trigger enabled efficient fraction collection of the target degradation products using the ACQUITY QDa II Detector.

References

1. United States Pharmacopeia (*USP), General Chapter* <1086>, “Impurities in Drug Substances and Drug Products” *USP* 40 and *PF* 41(3) (Rockville, MD, May–June 2015).
2. Dhangar KR, Jagtap RB, Surana SJ, Shirkhedkar AA. Review Article Impurity Profiling of Drugs Towards Safety and Efficacy: Theory and Practice. Journal of the Chilean Chemical Society, 62:2 (2017).
3. Maziarz M, Cleary RP, Shave D, Harden SN, Rainville PD, Xia J. Isolation of a Degradation Product of Ranitidine Hydrochloride Using the Waters Analytical Scale Purification System. Waters Application Note 720008785, 2025.
4. Waters Preparatory ODB Columns Calculator, https://www.waters.com/waters/promotionDetail.htm?id=134713448&locale=101

Arc, ACQUITY, QDa, XBridge, BEH, ODB, Empower, MassLynx, FractionLynx are trademarks of Waters Technologies Corporation.

TO DOWNLOAD A COPY OF THIS POSTER, VISIT WWW.WATERS.COM/POSTERS

720009084EN
©2025 Waters Corporation