Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

A Quality by Design (QbD) Based Method Development for the Determination of Impurities in a Peroxide Degraded Sample of Ziprasidone

Mia Summers and Kenneth J. Fountain
Waters Corporation, 34 Maple St., Milford, MA, USA

APPLICATION BENEFITS

- Faster, efficient separations and data management using an ACQUITY UPLC® H-Class with Column Manager and Solvent Select Valve in conjunction with Empower™ 2 software.
- Built-in method robustness using a Quality by Design (QbD) approach to generate a method that is amenable to continuous improvement without re-validation in the future.
- Streamlined method transfer from UPLC® to HPLC using the ACQUITY UPLC Columns Calculator and Method Transfer Kits facilitates transfer of methods to labs that may not equipped with UPLC.
- Significant time savings using a statistical design of experiments approach to method development to generate efficient sample sets that cover a wide experimental space.

WATERS SOLUTIONS

ACQUITY UPLC H-Class system
ACQUITY UPLC CSH C_{18} Columns
XSelect™ CSH™ C_{18} Columns
Empower 2 CDs
Fusion AE™ Method Development Software (S-Matrix)

KEY WORDS

Method development, UPLC, method transfer, Quality by Design, ziprasidone, degradation, CSH, ACQUITY UPLC Columns Calculator

INTRODUCTION

Method development can be a time-consuming process that can be repeated many times thoughout a drug development pipeline. Methods are commonly developed using a one-factor-at-a-time (OFAT) approach where one variable is changed sequentially until a suitable method is produced. This type of development may create an adequate method but provides a limited understanding of method capabilities and method robustness. Rather, a systematic screening approach that evaluates a number of stationary phases, pH ranges and organic modifiers provides a more thorough approach to method development. A Quality by Design (QbD) approach to method development uses statistical design of experiments (DoE) to develop a robust method 'design space'. The design space defines the experimental region in which changes to method parameters will not significantly affect the results. This approach builds-in robustness to the method as the method is being developed[1].

A better understanding of the overall method capabilities and limitations in development ensures a greater chance of successful downstream method validation, transfer and routine use. Software-driven method development affords considerable time savings for the scientist and the use of QbD can produce a significantly more robust and quality submission to regulatory authorities.

In this application note, a QbD approach to method development and subsequent method transfer to HPLC is presented on a forced degradation sample of ziprasidone, an anti-psychotic drug. Method development was performed using an ACQUITY UPLC H-Class system equipped with a column manager and solvent select valve to allow for automated exploration of a wide range of conditions, while obtaining efficient separations with shorter chromatographic run times. Fusion AE Method Development software was used in conjuction with Empower 2 to facilitate a more comprehensive QbD approach to method development.

EXPERIMENTAL

ACQUITY UPLC H-Class Conditions

Mobile phase:

A: Acetonitrile

B: Methanol

D1: Water with 0.1% Formic Acid (pH 2.5)

D2: Water with 0.1% Ammonium Hydroxide (pH 10.5)

Columns (All 2.1 x 50 mm, 1.7 µm)

1. ACQUITY UPLC CSH C_{18}
2. ACQUITY UPLC CSH Fluoro-Phenyl
3. ACQUITY UPLC BEH Shield RP18
4. ACQUITY UPLC HSS C_{18} SB
5. ACQUITY UPLC HSS T3
6. ACQUITY UPLC HSS Cyano

Needle Wash:	10:90 Water:Methanol
Sample Purge:	90:10 Water:Methanol
Seal Wash:	90:10 Water:Methanol
Detection:	UV at 254 nm

SCREENING (PHASE 1)

Flow Rate:	0.6 mL/min
Injection Volume:	2 µL
Column Temp.:	30°C
Gradient Time:	5 min
Variables:	stationary phase, mobile phase, gradient endpoint % organic, mobile phase pH 2.5 to 10.5

OPTIMIZATION (PHASE 2)

Column:	ACQUITY UPLC CSH C_{18}, 2.1 x 50 mm, 1.7 µm
Mobile Phase:	A: Acetonitrile
	D1: Water with 0.1% Formic Acid (pH 2.5)
Gradient endpoint:	87.5% Acetonitrile
Variables:	gradient time, column temperature, injection volume, flow rate
Data Management:	Empower 2 CDS Fusion AE Method Development Software (S-Matrix)

SAMPLE PREPARATION

Ziprasidone peroxide degradation sample:

To 0.4 mg/ml ziprasidone in 50:50 water:methanol, add one equal volume of 3% hydrogen peroxide solution in water, heat at 80°C for 30 min. Dilute to 0.1 mg/mL final concentration with water.

RESULTS AND DISCUSSION:

Phase 1: Screening

Method development was performed using an ACQUITY UPLC H-Class system, Empower 2 and Fusion AE Method Development software. The H-Class was equipped with a 6-position column manager and a solvent select valve to enable full method development capability in one system. The initial screening varied column chemistries having CSH, BEH and HSS base particles for maximum selectivity. Organic modifier (acetonitrile or methanol) was screened varying the gradient endpoint from 50% to 100% organic, over a mobile phase pH range from 2.5 to 10.5.

Using these parameters, an experimental design was generated within Fusion AE, including randomization and replicate injections. The design generated encompassed the entire knowledge space defined by the constants and variables entered during the experimental setup. A partial factorial statistical design was selected by the software to obtain the maximum amount of information with the least number of experimental runs. The experimental design was transmitted to Empower2 software where all methods, method sets and sample sets were automatically generated and ready to run.

After initial integration and processing, results from the screening analysis for ziprasidone were imported back into Fusion AE and processed to generate an initial method for subsequent optimization. For the ziprasidone peroxide degradation sample, a water/acetonitrile gradient at pH 2.5 with an 87.5% acetonitrile gradient endpoint on a CSH C_{18} column was found to be optimal. The method developed is compatible with mass spectrometric detection and was directly transferred to LCMS to rapidly identify the ziprasidone forced-degradation products (Figure 1).

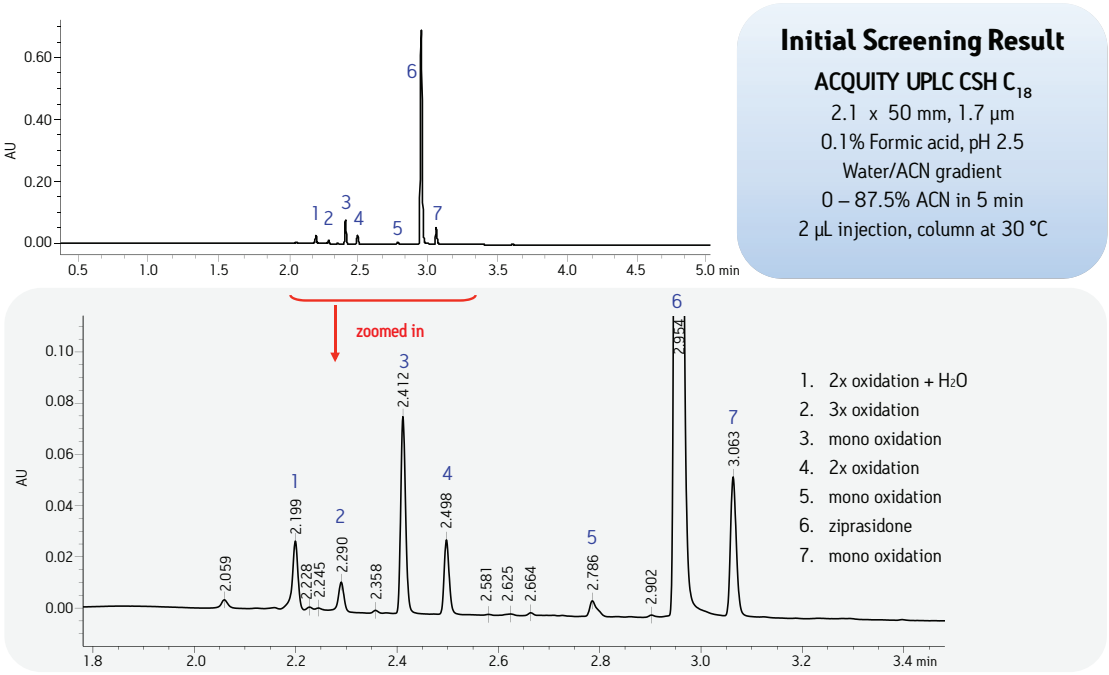

Figure 1. Initial method from screening experiments for ziprasidone peroxide degradation.

Phase 2: Method Optimization

The initial method was further optimized in a second experiment where secondary effectors such as column temperature, injection volume, gradient slope (modified using gradient time) and flow rate were varied. A new experimental design was generated by Fusion AE and new methods and sample sets were automatically created within Empower 2.

After processing data in Fusion AE, the final optimized method was generated, demonstrating the method that best meets the success criteria defined by the user. In the case of the ziprasidone peroxide degradation separation (Figure 2), an improvement in the tailing of peak 2 is seen along with better resolution of baseline impurity peaks and a newly resolved impurity is observed at 2.075 min.

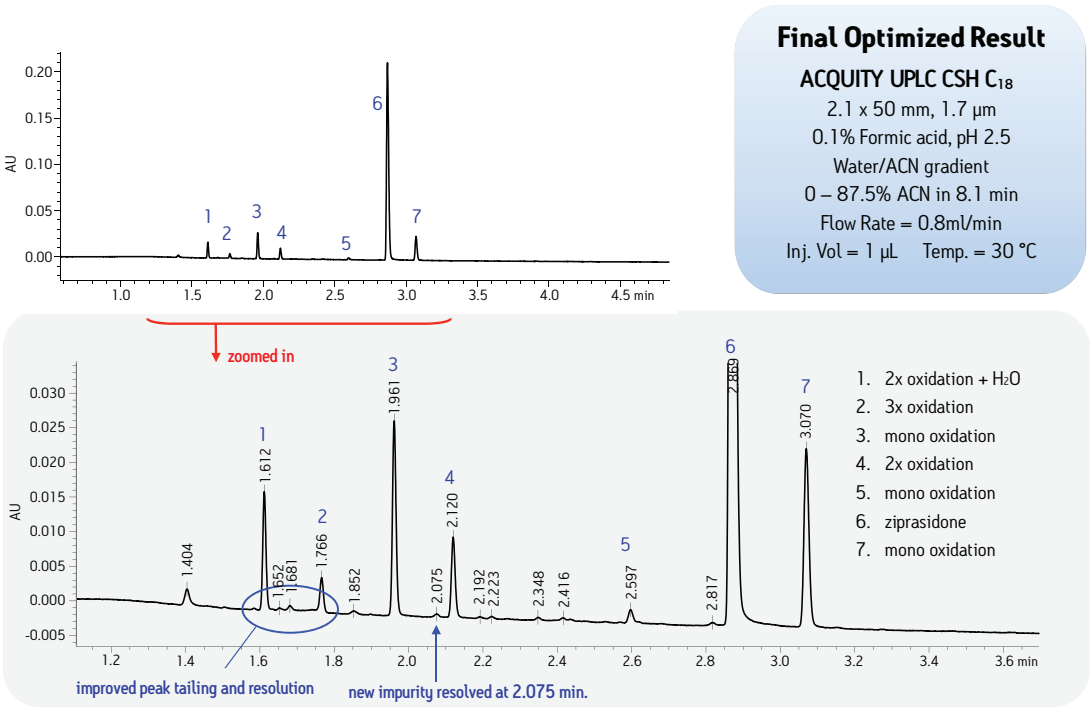

Figure 2. Final optimized method for ziprasidone peroxide degradation showing improved peak tailing and resolution.

Multi-dimensional plots in Fusion AE facilitates visualization of the effect of each factor on the separation (Figure 3). The white region of the 2D contour plot depicts the design space, which defines the robust region of the method where results are within designated criteria. By changing the factors on each axis, the design space can be explored in detail and method robustness can be fully understood.

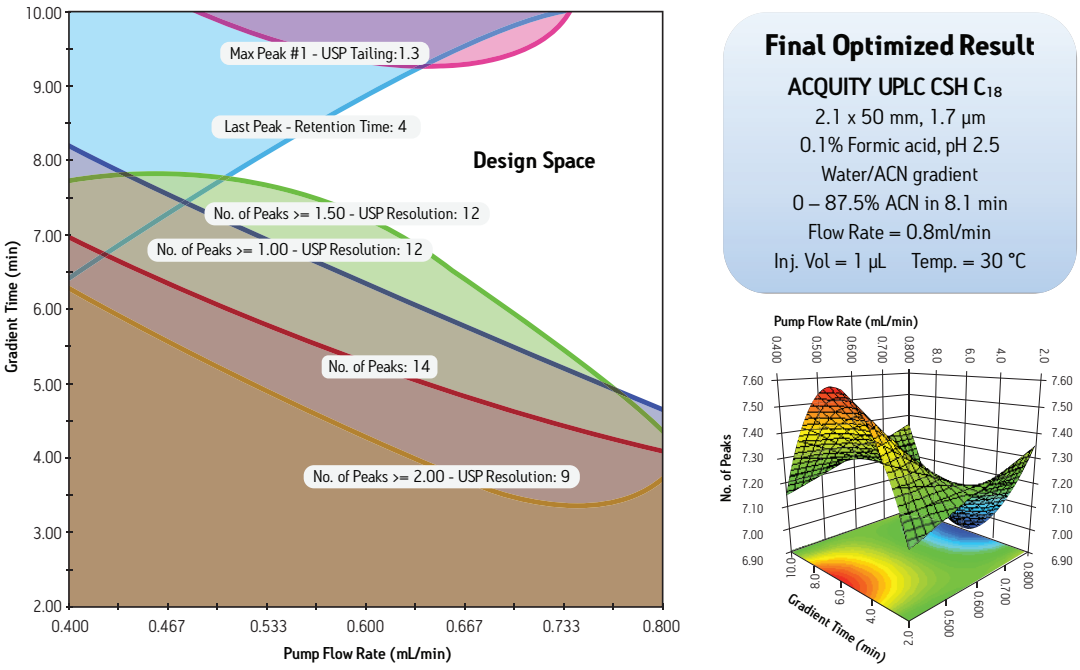

Figure 3. The design space region showing the independent effects of gradient time and pump flow rate on method success. Data can also be visualized in 3D plots as shown.

Method Transfer

The UPLC method developed using Fusion AE software was transferred to HPLC to demonstrate transferability from a method development laboratory to a quality control (QC) laboratory that might not be equipped with UPLC. Method transfer was performed using the ACQUITY UPLC Columns Calculator and Method Transfer Kit, scaling for particle size[2]. The method was scaled from the ACQUITY UPLC CSH C_{18} 2.1 x 50 mm 1.7 µm particle column to the corresponding XSelect CSH C_{18} 4.6 x 150 mm 5 µm HPLC column. A comparison of the UPLC and HPLC separation demonstrates that the peak profile and resolution is maintained when scaling to HPLC conditions from method development on UPLC.

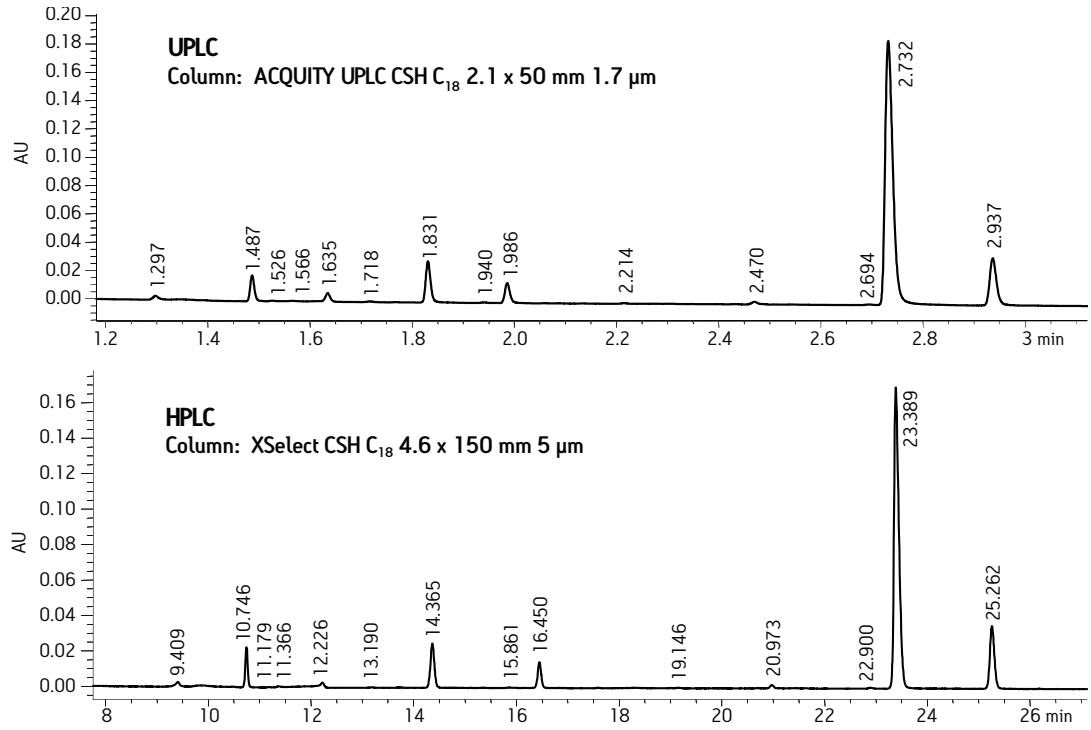

Figure 4. Method transfer from UPLC to HPLC for ziprasidone peroxide degradation.

CONCLUSIONS:

- A robust method for ziprasidone peroxide degradation was developed in two days using a Quality by Design approch on an ACQUITY UPLC H-Class system running Empower 2 and Fusion AE Method Development software.
- QbD method development software in conjunction with ACQUITY UPLC H-Class system automation allows for rapid screening and optimization across a wide range of column chemistries, mobile phases and pH ranges, while evaluating the effects of secondary factors such as column temperature, flow rate, injection volume and gradient slope on the separation.
- A comprehensive method development experiment can be rapidly performed by combining fast separations using UPLC with efficient experimental designs by Fusion AE Method Development software.
- The UPLC method developed for ziprasidone peroxide degradation was transferred to HPLC in one step using a Method Transfer Kit and ACQUITY UPLC Columns Calculator, demonstrating ease of transfer of developed methods to labs that may not be equipped with UPLC.

REFERENCES:

1. **Alden PG, Potts W, Yurach D. A QbD with Design-of-Experiments Approach to the Development of a Chromatographic Method for the Separation of Impurities in Vancomycin. Waters Application Note 70003719EN. 2010 Sept.**
2. **Jones MD, Alden P, Fountain KJ, Aubin A. Implementation of Methods Translation between Liquid Chromatography Instrumentation. Waters Application Note 720003721EN. 2010 Sept.**

Waters

THE SCIENCE OF WHAT'S POSSIBLE.™

Waters Corporation
34 Maple Street
Milford, MA 01757 U.S.A.
T: 1 508 478 2000
F: 1 508 872 1990
www.waters.com