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Validation of a Method for the Separation of Ziprasidone and Its Degradants using Empower 2 with Method Validation Manager (MVM)

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APPLICATION BENEFITS

- Easy monitoring of the validation study and complete traceability of data in one location, instead of error-prone data tracking using multiple spreadsheets and paper trails
- Efficient sample set designs save sample preparation and instrument time
- Robustness testing analyzing multiple factors at a time results in considerable time savings when compared to a one-factor-at-a-time (OFAT) approach
- Testing multiple column batches made with different stationary-phase batches ensures long-term method ruggedness

WATERS SOLUTIONS

- ACQUITY UPLC® H-Class system
- ACQUITY UPLC CSH™ C_{18} and XSelect™ CSH C_{18} columns
- Method Validation Kits (MVKs)
- Empower™ 2 with Method Validation Manager (MVM) software

KEY WORDS

Method validation, UPLC, batch, reproducibility, ziprasidone, degradation, CSH

INTRODUCTION

Method validation is an essential part of analytical method development in a regulated environment. The process of running a validation study, from defining tests, specifications and acceptance criteria to sample testing and analysis, is a data-intensive and time-consuming process. The use of additional spreadsheets and paper print-outs to process data and track results are error-prone processes that can be remedied using a validation software package such as Empower 2 with Method Validation Manager (MVM).[1]

In this application note, the validation of a method developed for the separation of ziprasidone and associated impurities from a forced degradation is demonstrated using Empower 2 with Method Validation Manager (MVM). While a variety of tests can be set-up using MVM, the validation tests performed in this example include accuracy, linearity, robustness, repeatability, limit of detection (LOD) and limit of quantitation (LOQ) determinations. Intermediate precision was also tested using a Method Validation Kit, to ensure reliability of the method across three different batches of the same column chemistry. The use of efficient sample set designs, along with automated data processing using Empower 2 with MVM, allowed these six validation tests to be performed and processed in one day with simple tracking of the validation study.

EXPERIMENTAL

ACQUITY UPLC H-Class Conditions

Mobile Phase:	A: acetonitrile
	D1: water with 0.1% formic acid (pH 2.5)
Columns:	ACQUITY UPLC CSH C_{18}, 2.1 x 50 mm, 1.7 µm, part number 186005296
Needle Wash:	10:90 water:methanol
Sample Purge:	90:10 water:methanol
Seal Wash:	90:10 water:methanol
Detection:	UV at 254 nm
Flow Rate:	0.8 mL/min
Injection Volume:	1 µL
Column Temp.:	30 °C
Gradient Time:	1 to 45% acetonitrile over 4.3 min., re-equilibrate at starting conditions
Data Management:	Empower 2 CDS with Method Validation Manager (MVM)

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 minutes. Dilute to 0.1 mg/mL final concentration with water.

RESULTS AND DISCUSSION

The method for the ziprasidone peroxide degradation separation was previously developed using a Quality by Design (QbD) approach on an ACQUITY UPLC H-Class system running Empower 2 and Fusion AE Method Development Software.[2] Since all components eluted before 4 minutes in the Fusion developed method, the original 12-minute run time was shortened to 6 minutes by adjusting the gradient from 0 to 87.5% acetonitrile over 8.1 minutes (original method) to 1% to 45% acetonitrile over 4.3 minutes. The slope of the original gradient was kept constant to maintain the same separation selectivity in both methods. Standards for the impurities generated from the ziprasidone peroxide degradation were not readily available and therefore, a full impurities method validation was not performed. Instead, select validation tests were performed on the ziprasidone active pharmaceutical ingredient (API) and four impurities from the ziprasidone peroxide degradation separation are shown in Figure 1 to demonstrate the utility of Empower 2 with MVM.

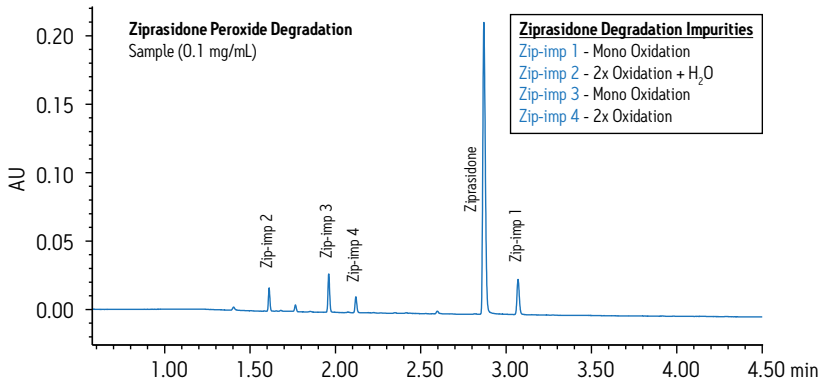

Figure 1. The ziprasidone peroxide degradation separation developed using Quality by Design (QbD).

Accuracy, Linearity and Repeatability, LOD/LOQ

Accuracy was evaluated using triplicate preparations at five levels (80, 90, 100, 110, 120%) of the target concentration of ziprasidone API (0.1 mg/mL). Linearity was assessed using the same range as the accuracy testing protocol. Repeatability was tested using six preparations of 100% target concentration of ziprasidone API. Ziprasidone impurities were not evaluated for these tests as appropriate standards were not readily available.

The acceptance criteria for accuracy, linearity and repeatability tests were specified when setting up the validation protocol in MVM. One sample set was written in Empower 2 to generate data for all three tests, enabling MVM to use the results from one sample set run to complete testing for accuracy, linearity and repeatability.

Based on the specifications defined in the validation protocol, the method was found to be accurate and linear within the range tested and the repeatability of the method was well within the established acceptance criteria (Table 1). Using the residual standard deviation from the linearity curve, LOD and LOQ were also calculated for ziprasidone API. The use of efficient sample sets and MVM allowed all of these tests to be completed in one sample set run, greatly minimizing sample preparation and instrument time. Automated data processing using Empower 2 with MVM allowed rapid determination of the status and results of each test, without the need to export data to a separate spreadsheet for manual analysis.

Test	Acceptance Criteria	Ziprasidone	Result
Accuracy	% Recovery 95- 105%	97.6-101.5	Pass
Linearity	R_2 > 0.99	0.993	Pass
	Residuals <2% RSD	1.6	
Repeatability	Ret Time <2% RSD	0.1	Pass
	% Area <2% RSD	1.1	Pass
LOD	-	0.004 mg/mL	
LOQ	-	0.013 mg/mL	

Table 1. Accuracy, linearity, repeatability, limit of detection (LOD), and limit of quantitation (LOQ) results for ziprasidone.

Robustness

Method robustness tests were configured in MVM using design of experiments (DoE). Based on the factors entered, MVM generates an efficient experimental design that will analyze multiple factors at a time, resulting in a significant time savings compared to a one-factor-at-a-time approach. The factors analyzed in this study included flow rate (0.7-0.9 mL/min), column temperature (30 to 60 °C) and injection volume (0.5-1.5 µL). Robustness testing was performed in eight runs using a full factorial experimental design.

Method robustness was evaluated for the ziprasidone API and the four ziprasidone degradation products based on peak retention time RSD and % area RSD (Table 2). The method is shown to be robust within the criteria defined in the validation protocol for all three factors evaluated. The use of experimental design and automated processing of multiple factors in MVM greatly facilitates robustness testing in validation studies.

Test	Acceptance Criteria	Ziprasidone	Zip-imp 1	Zip-imp 2	Zip-imp 3	Zip-imp 4	Result
Robustness	Ret Time <10% RSD	5.6	5.3	8.2	7.5	7.1	Pass
	% Area <2.5% RSD	1.2	0.7	2.1	1.6	1.5	

Table 2. Robustness results for ziprasidone and four impurities.

Intermediate Precision

The intermediate precision (or ruggedness) of the method was evaluated across column batches using a Method Validation Kit (MVK). MVKs consist of three different batches of the same column chemistry, hence maximizing the analytical variability that might be seen using different batches of columns. A comparison of the ziprasidone peroxide degradation separation on three different batches of ACQUITY UPLC CSH C_{18} columns in the kit is shown in Figure 2. MVKs provide easy accessibility to three different batches of the same column and their use in both method development and validation promotes analytical method ruggedness as columns are replaced over the lifetime of the method.

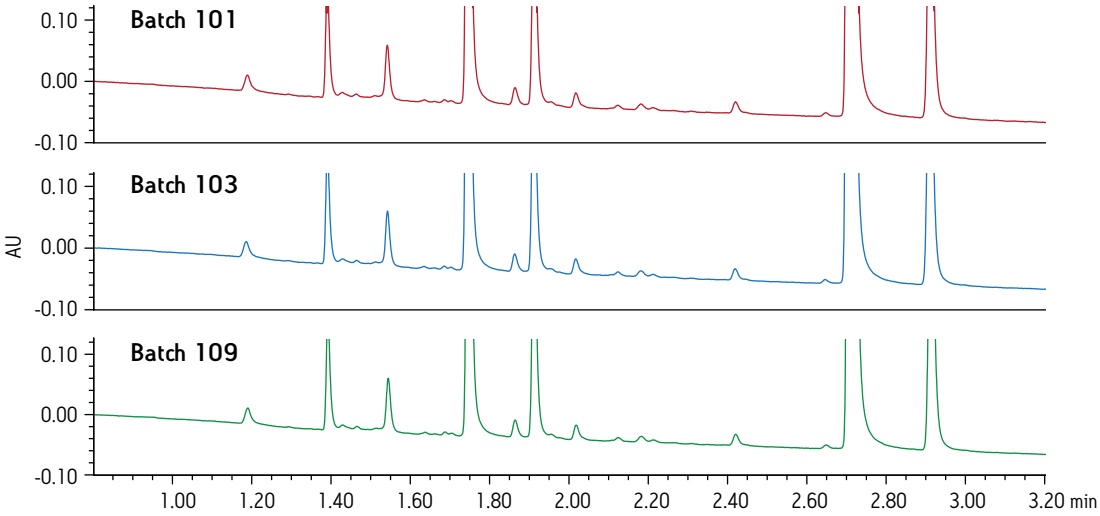

Figure 2. Zoomed-in comparison of the ziprasidone peroxide degradation separation on three different batches of CSH C_{18} using a Method Validation Kit (MVK).

Triplicate preparations of the ziprasidone peroxide degradation sample were analyzed on each of three batches of columns from the ACQUITY UPLC CSH C_{18} MVK. The intermediate precision criteria of retention time RSD and peak area RSD were evaluated for ziprasidone and each of the four impurities (Table 3). The results were found to be within defined acceptance criteria, demonstrating the ruggedness of the method across different batches of columns.

Test	Acceptance Criteria	Ziprasidone	Zip-imp 1	Zip-imp 2	Zip-imp 3	Zip-imp 4	Result
Intermediate Precision	Ret Time <2% RSD	1.1	1.1	1.1	0.9	0.8	Pass
	Peak Area <5% RSD	1.7	2.1	2.6	3.8	4.9	

Table 3. Intermediate precision results for ziprasidone and four impurities, testing three column batches using a Method Validation Kit.

CONCLUSIONS

- Software-based validation approaches such as Empower 2 with MVM use efficient sample sets and experimental designs, allowing validation testing to be performed much faster than setting up and running one experiment at a time. For the validation of the ziprasidone impurities separation, six validation tests were performed and results were processed all in one day.
- Intermediate precision evaluated using Method Validation Kits help ensure long-term ruggedness of the method across three different batches of columns, reducing the risk of reproducibility issues and downstream re-validation on a method developed using only one column or one batch of packing material.
- Method validation performed using Empower 2 with MVM requires no additional software to validate. MVM eliminates the need to transfer data to external spreadsheets for manual analysis, greatly reducing transcription error. All samples are run, processed and compiled in one location, facilitating data tracking, improving data security, and increasing audit confidence.

REFERENCES

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2. Summers, M., Fountain, K.J. *"A Quality by Design (QbD) Based Method Development for the Determination of Impurities in a Peroxide Degraded Sample fo Ziprasidone*," Waters Application Note [2011], Part Number 720004072EN.

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