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Application Note

Transfer of USP Methods for Impurities Analysis of Ziprasidone HCl between HPLC Systems and to UPLC

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Abstract

Transferring compendial HPLC methods between systems can be challenging. Many of the USP monographs utilize diluents with a high concentration of organic solvents for the preparation of standard and sample solutions. While strong solvent diluents are common for HPLC applications, they often prove unsatisfactory for analyses performed on modern systems that feature minimized dispersion.

In this study, the transfer of two USP compendial methods for impurities analysis of ziprasidone HCl from HPLC to UPLC is demonstrated with a straightforward way to address this potential issue.

Benefits

- Method transferability between different systems and different sites
- 63% reduction in run time when using UPLC
- 93% savings in mobile phase consumption when using UPLC
- Reduced cost for solvent and waste disposal

Introduction

U.S. Pharmacopeia (USP) compendial methods are routinely adopted by pharmaceutical companies for testing raw materials and finished products. Successful implementation of the USP methods and transferability between instruments are key steps to enhance throughput for routine analysis. Effective method transfer generates identical results for the same analysis independent of the laboratory, instrument, and the resources for a specific method.

By ensuring successful lab-to-lab method transferability, companies can replicate methods at additional sites or with partners such as contract research or manufacturing organizations (CROs and CMOs). Transferring an HPLC-based USP method to UPLC Technology offers such organizations the additional opportunity to achieve productivity goals by reducing analysis time while ensuring reliable, high-quality chromatographic separations that are the basis for decisions about product quality. UPLC Technology offers QC and manufacturing facilities significant advantages in terms of increased throughput, improved quality, and reduced costs.

Transferring compendial HPLC methods between systems can be challenging. Many of the USP monographs utilize diluents with a high concentration of organic solvents for the preparation of standard and sample solutions. While strong solvent diluents are common for HPLC applications, they often prove unsatisfactory for analyses performed on modern systems that feature minimized dispersion.

In this study, the transfer of two USP compendial methods for impurities analysis of ziprasidone HCl[1] from HPLC to UPLC is demonstrated. The use of strong solvent as a sample diluent can be a barrier when transitioning a method to a low-volume system, depending on the injection volume. Presented here is a straightforward way to address this potential issue.

The success of the method transfer between different HPLC systems and conversion to UPLC was measured by evaluating the system suitability requirements listed in the USP monograph for ziprasidone HCl. Success of the transfer was further demonstrated by running the methods on the same instrument configurations in a laboratory located in a different country, where identical results were achieved.

Experimental

Sample description

All solutions (Table 1) were prepared in methanol/water/ concentrated HCl at a composition of 20:5:0.01 to comply with the impurities methods defined in the USP monograph for ziprasidone HCl. Since the USP does not list a sample preparation protocol for the ziprasidone HCl capsules, the drug substance sample preparation was used with one modification. Sample solutions were filtered through 0.2-µm PTFE syringe filters to remove any particulates.

Solutions

Early-eluting peaks	Late-eluting peaks
System suitability solution:	System suitability solution:
0.24 mg/mL of ziprasidone HCl	0.24 mg/mL of ziprasidone HCl
0.5 µg/mL of related compound A	0.8 µg/mL of related compound C

Early-eluting peaks	Late-eluting peaks
0.8 µg/mL of related compound B	0.8 µg/mL of related compound D
Standard solution:	Standard solution:
0.5 µg/mL of related compound A	0.8 µg/mL of related compound C
0.8 µg/mL of related compound B	0.8 µg/mL of related compound D
Sample solution:	Sample solution:
0.4 mg/mL of capsule content	0.45 mg/mL of capsule content

Table 1. Standard and sample solutions composition for impurities analysis of ziprasidone HCl.

System control, data acquisition, and analysis

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HPLC conditions for early-eluting impurities method

LC systems:	Alliance 2695 HPLC with 2489 UV/Visible Detector ACQUITY UPLC H-Class with TUV Detector
Column:	XBridge C_8 4.6 x 150 mm, 5 µm
Column temp.:	40 °C
Sample temp.:	10 °C
Injection volume:	20 µL
Flow rate:	1.5 mL/min

Mobile phase:	2:3 methanol/buffer. Buffer: 50 mM potassium phosphate monobasic, pH 3.0 adjusted with phosphoric acid
Separation mode:	Isocratic
Wash solvents:	50:50 water/methanol
Detection:	UV, 229 nm

HPLC conditions for late-eluting impurities method

LC systems:	Alliance 2695 HPLC with 2489 UV/Visible Detector ACQUITY UPLC H-Class with TUV Detector
Column:	XBridge C_8 4.6 x 150 mm, 5 µm
Column temp.:	35 °C
Sample temp.:	10 °C
Injection volume:	20 µL
Flow rate:	1.0 mL/min
Mobile phase:	11:1:8 acetonitrile/methanol/buffer Buffer: 50 mM potassium phosphate monobasic, pH 6.0 adjusted with 5N potassium hydroxide
Separation mode:	Isocratic
Wash solvents:	50:50 water/methanol
Detection:	UV, 229 nm

UPLC conditions for early-eluting impurities method

LC system:	ACQUITY UPLC H-Class with TUV Detector
Column:	ACQUITY UPLC BEH C_8, 2.1 x 50 mm, 1.7 µm
Column temp.:	40 °C
Sample temp.:	10 °C
Injection volume:	1.4 µL
Flow rate:	0.313 mL/min
Mobile phase:	2:3 methanol/buffer Buffer: 50 mM potassium phosphate monobasic, pH 3.0 adjusted with phosphoric acid
Separation mode:	Isocratic
Wash solvents:	50:50 water/methanol
Detection:	UV, 229 nm

UPLC conditions for late-eluting impurities method

LC system:	ACQUITY UPLC H-Class with TUV Detector
Column:	ACQUITY UPLC BEH C_8, 2.1 x 50 mm, 1.7 µm
Column temp.:	35 °C
Sample temp.:	10 °C
Injection volume:	1.4 µL

Flow rate:	0.208 mL/min
Mobile phase:	11:1:8 acetonitrile/methanol/buffer Buffer: 50 mM potassium phosphate monobasic, pH 6.0 adjusted with 5N potassium hydroxide
Separation mode:	Isocratic
Wash solvents:	50:50 water/methanol
Detection:	UV, 229 nm

USP system suitability criteria for early-eluting method:

For five replicate injections of system suitability solution

- Resolution between ziprasidone and ziprasidone related compound B: Not less than (NLT) 1.5
- Relative standard deviation (RSD) for ziprasidone related compound B: Not more than (NMT) 10%

USP system suitability criteria for late-eluting method:

For five replicate injections of system suitability solution

- Resolution between ziprasidone and ziprasidone related compound C: NLT 6.0
- RSD for ziprasidone related compound C: NMT 10%

Results and Discussion

Managing system volume differences

One of the challenges of migrating a USP monograph from legacy to modern LC technologies is the impact of technology improvements in managing system volume and dispersion that may cause distorted peaks. The volume of an LC system in front of the injector will impact the time required to equilibrate the method to the initial conditions. The system volume after the injector is related to the system dispersion, which mixes sample with the mobile phase. In this work, we investigate these relationships and determine an effective

way to adjust for different volumes.

As an example, injecting sample dissolved in a strong organic diluent onto systems with a small post-injector volume can yield a less focused injecton, often evidenced by fronting. Peaks with lower retention factor (k') eluting isocratically are typically observed to be more distorted compared to the later eluting peaks with larger k'. Methods with a gradient elution are also susceptible but less affected by the strong organic diluents, due to peak focusing at the head of the column.

Method transfer of USP HPLC method

The USP monograph designates using an L7 column for the ziprasidone HCl early and late impurities methods and suggests using a Zorbax RX-C_8 Column. Using the Waters Reversed-Phase Column Selectivity Chart (www.waters.com/selectivitychart), a Waters XBridge C_8 Column was chosen. The standard and sample solutions were prepared in diluent containing 80% methanol. The compendial method for the early eluting impurities was run as described on the Alliance HPLC System (Figure 1A), and on the ACQUITY UPLC H-Class System (Figure 1B) using the same XBridge C_8 Column and the same mobile phase. Distortion of the peaks was observed when the system suitability solution was injected on the ACQUITY UPLC H-Class System.

Several experiments were designed to investigate and solve the issue of observed peak distortion. It was hypothesized that the peak distortion was due to a high concentration of organic solvent. These experiments included the following:

1. Reducing the injection volume.
2. Reducing the concentration of organic solvent in sample diluent.
3. Increasing the post-injector volume of the UPLC system.

The USP allows reduction of the injection volume as long as the precision and the detection limits are acceptable.[2] While reducing the injection volume improved peak symmetry and resolution, it decreased sensitivity (Figure 2). For this reason, injection volume could not be decreased to mitigate the distorted peaks.

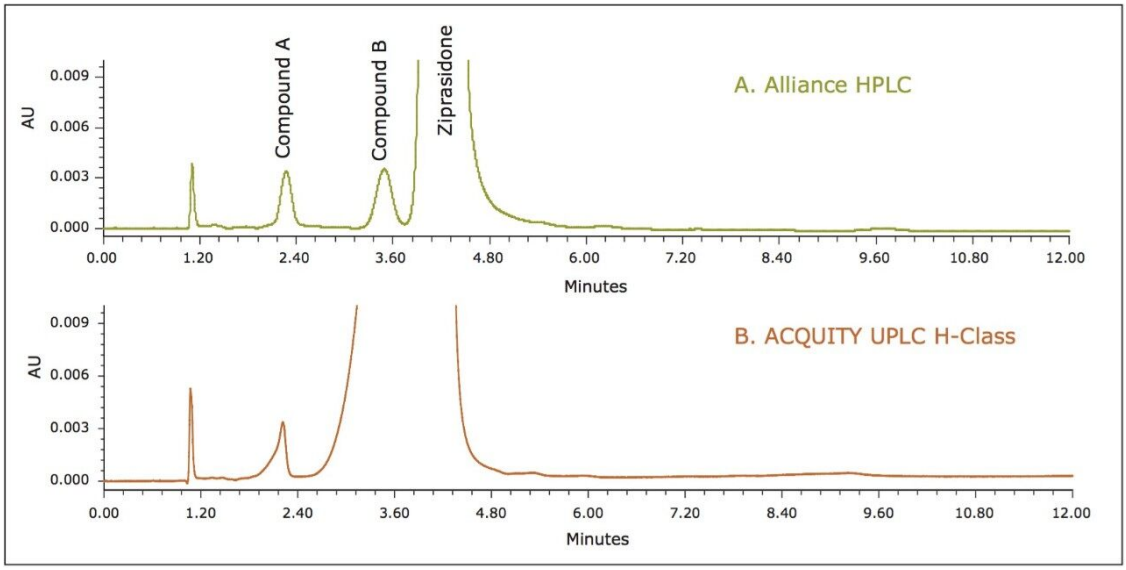

Figure 1. HPLC data of the system suitability solution for ziprasidone HCl early-eluting impurities analysis.

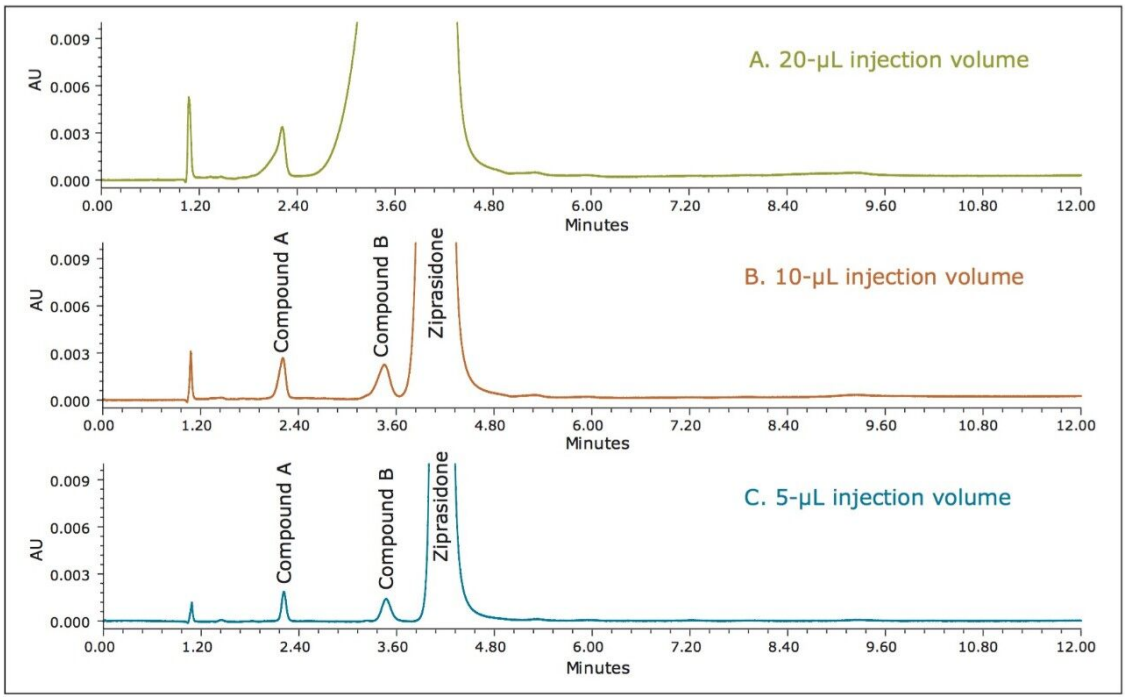

Figure 2. Injection volume study to investigate HPLC analysis performed on an ACQUITY UPLC H-Class System. System suitability solution was prepared in diluent containing 80% methanol.

Reducing the composition of methanol in the diluent to 70% enhanced the chromatographic separation

(Figure 3). However, modification of the sample diluent is not recommended by the USP.

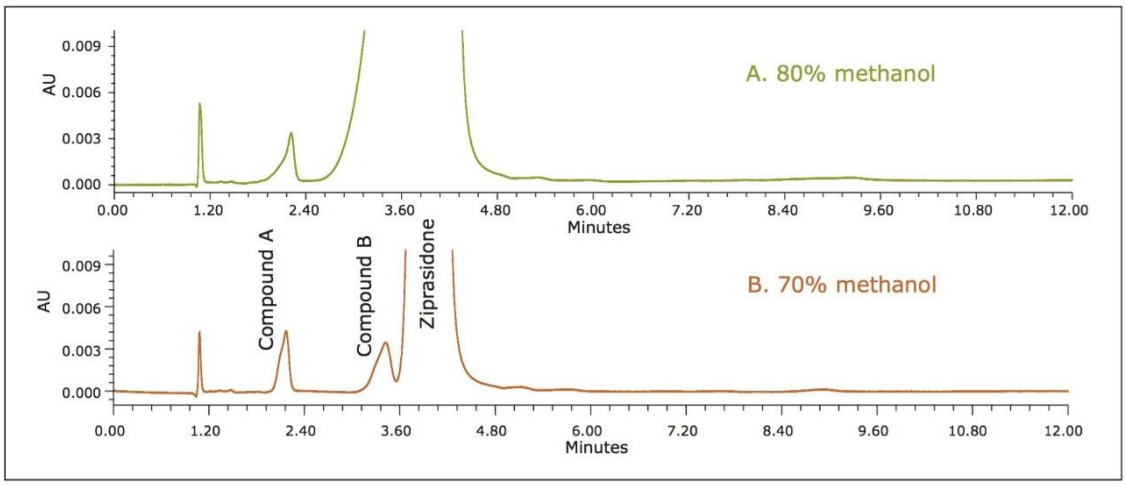

Figure 3. Reducing solvent concentration in sample diluent to investigate HPLC analysis performed on an ACQUITY UPLC H-Class System. The injection volume was 20 µL.

Increasing system volume between the injector and the column inlet with 50-µL tubing significantly improved peak shape and resolution between all the peaks, conforming to allowable modifications documented by the USP (Figure 4).

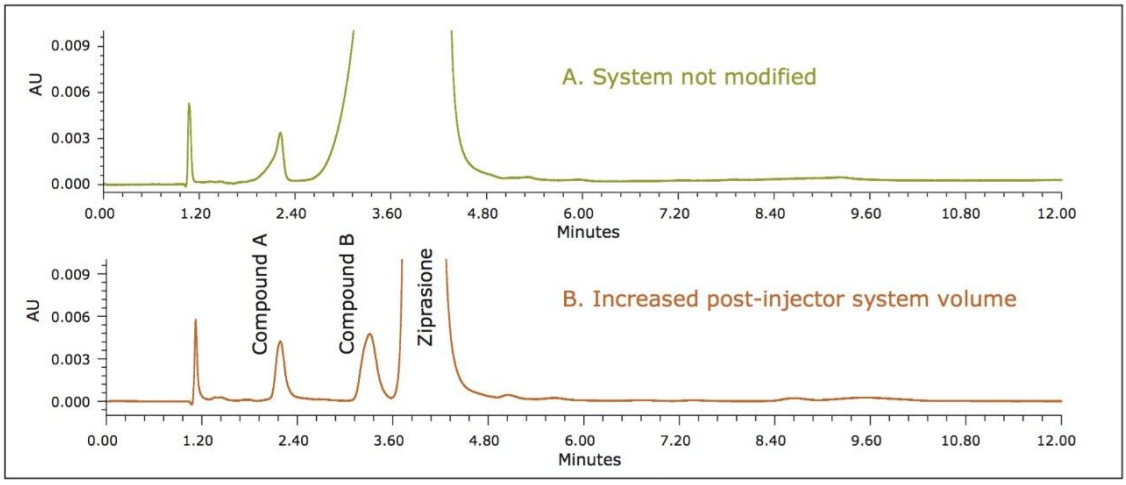

Figure 4. Increasing post-injector volume of an ACQUITY UPLC H-Class System to investigate mitigation of the strong solvent effects. System suitability solution was prepared in diluent containing 80% methanol and injected at 20 µL.

Finally, increasing the volume of the UPLC system after the injector allows the diluent to mix with the mobile phase before it enters the column, improving peak symmetry and resolution.

By increasing the post-injector volume, the compendial HPLC methods for the early- and late-eluting impurities could be successfully run on the ACQUITY UPLC H-Class System. Overall, transferability of the compendial methods between LC systems with different extra-column volume, specifically the Alliance HPLC and ACQUITY UPLC H-Class systems, was successful (Figures 5 and 6).

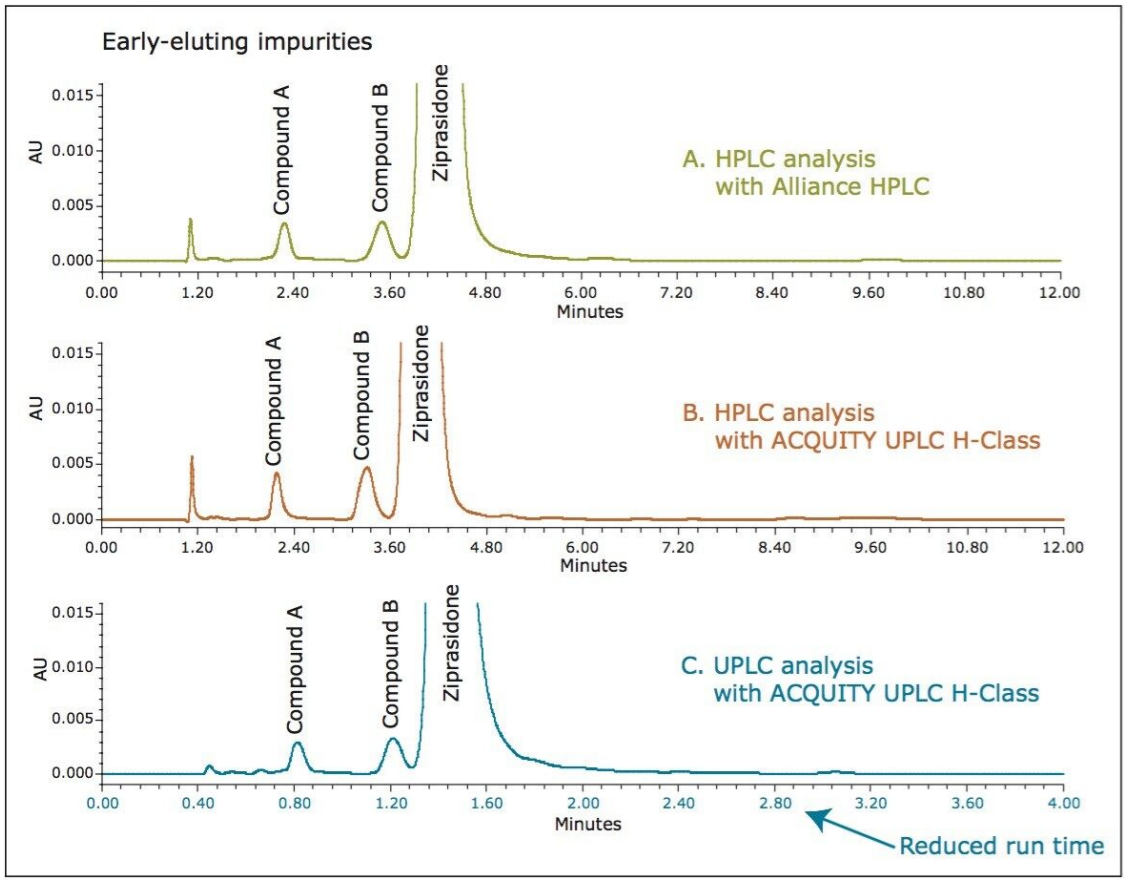

Figure 5. Chromatographic data of ziprasidone HCl early-eluting impurities method.

A. HPLC analysis acquired using Alliance HPLC System.

B. HPLC analysis acquired using ACQUITY UPLC H-Class System with an increased post-injector volume.

C. UPLC analysis acquired using ACQUITY UPLC H-Class System.

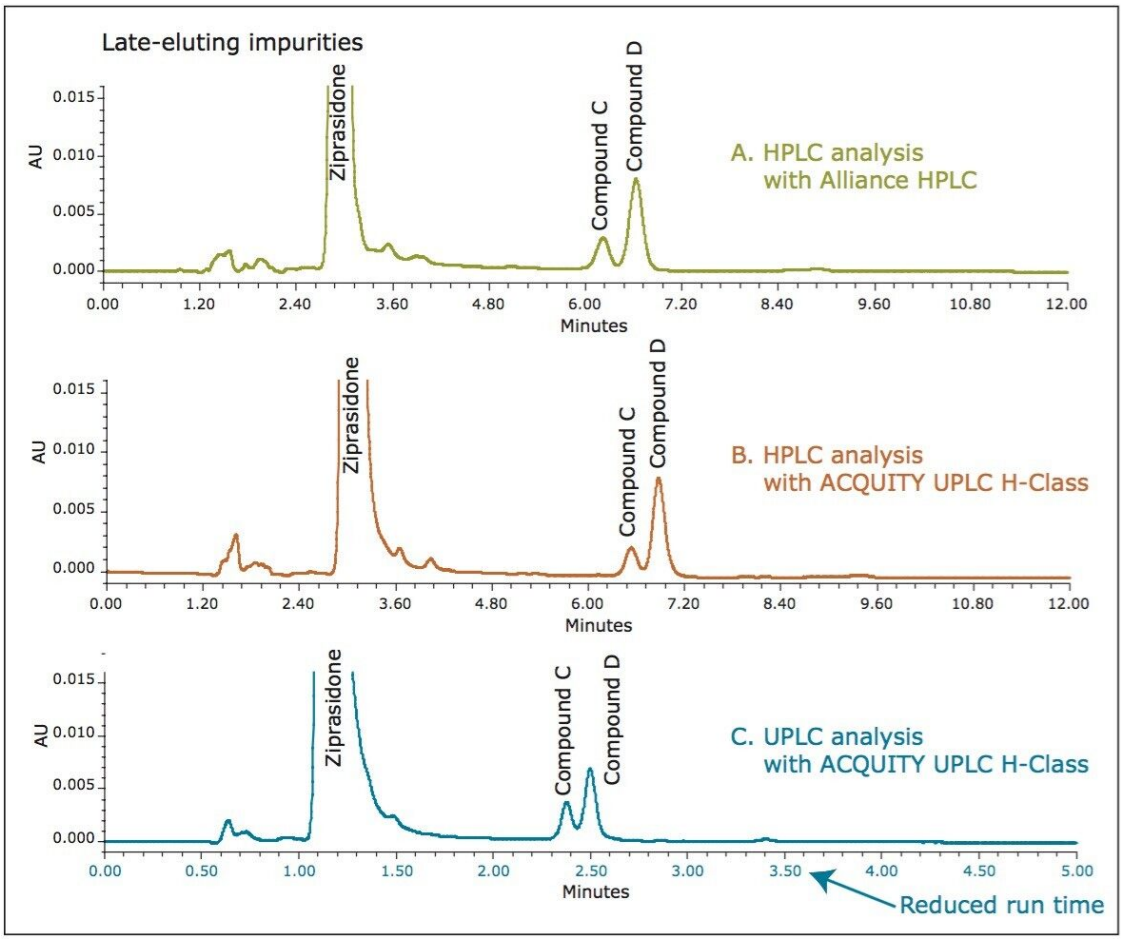

Figure 6. Chromatographic data of ziprasidone HCl late-eluting impurities method.

A. HPLC analysis acquired using Alliance HPLC System.

B. HPLC analysis acquired using ACQUITY UPLC H-Class System with an increased post-injector volume.

C. UPLC analysis acquired using ACQUITY UPLC H-Class System.

Finally, the system suitability results for the five replicate injections of the system suitability solution met the requirements listed in the USP monograph for ziprasidone HCl (Tables 2 and 3).

Parameter	USP criteria	HPLC analysis		UPLC analysis
		Alliance HPLC	ACQUITY UPLC H-Class	
Resolution between ziprasidone and related compound B	NLT 1.5	1.8	1.9	1.7
%RSD for related compound B	NMT 10%			
■ Retention times		0.2%	0.0%	0.1%
■ Peak areas		0.4%	0.1%	0.3%

Table 2. System suitability results for USP method transfer of the ziprasidone HCl early-eluting impurities method from Alliance HPLC to the ACQUITY UPLC H-Class System.

Parameter	USP criteria	HPLC analysis		UPLC analysis
		Alliance HPLC	ACQUITY UPLC H-Class	
Resolution between ziprasidone and related compound C	NLT 6.0	14.1	18.4	16.3
%RSD for related compound C	NMT 10%			
■ Retention times		0.0%	0.1%	0.0%
■ Peak areas		0.1%	0.7%	0.2%

Table 3. System suitability results for the USP method transfer of the ziprasidone HCl late-eluting impurities method from Alliance HPLC to the ACQUITY UPLC H-Class System.

Lab-to-lab method transfer

Finally, a lab-to-lab method transfer study was conducted using the compendial HPLC method for the earlyeluting impurities on the ACQUITY UPLC H Class System. Two different laboratories performed the test,including Waters laboratories in Milford, MA, USA and Guyancourt, Yvelines, France.

Reproducibility of the HPLC method acquired by both labor atories was demonstrated by comparing the system suitability results (Table 4). System suitability results acquired by both laboratories were comparable and met the requirements defined in the USP monograph for ziprasidone HCl.

Parameter	USP criteria	HPLC analysis on ACQUITY UPLC H-Class	
		Milford, MA (USA)	Guyancourt, Yvelines (France)
Resolution between ziprasidone and related compound B	NLT 1.5	1.9	1.6
%RSD for related compound B ■ Retention times ■ Peak areas	NMT 10%	0.0% 0.1%	0.1% 0.9%

Table 4. System suitability results for lab-to-lab transfer of the HPLC method for ziprasidone HCl early-eluting impurities on the ACQUITY UPLC H-Class System.

Conclusion

Two compendial HPLC methods for impurities of ziprasidone HCl were tested on two different HPLC systems and successfully transferred to UPLC. Increasing post-injector volume of the ACQUITY UPLC H-Class System was required to duplicate the chromatographic separation and meet the USP requirements for system suitability.

The ACQUITY UPLC H-Class System successfully replicated the analytical quality of the HPLC compendial methods. Excellent performance of both the HPLC and the UPLC methods demonstrate that the ACQUITY UPLC H-Class System is suitable for HPLC and UPLC applications.

- Implementing the ACQUITY UPLC H-Class System within quality control laboratories can reduce the costs of operation and maintenance by maximizing asset utilization and eliminating the need for multiple systems to perform HPLC and UPLC analyses.
- Conversion to UPLC technology can decrease overall costs and improve laboratory throughput and productivity for release testing of finished products.
- The UPLC methods for the required early- and late-eluting impurities tests provided a 67% and 58% reduction in run times over the corresponding HPLC methodologies.
- In addition to reduced analysis time, UPLC technology provides cost savings related to solvent and waste disposal. Consumption of mobile phase per UPLC injection, for the early and late impurities methods, is

reduced by 93% compared to the HPLC injection.

The reproducibility of the compendial HPLC method on the ACQUITY UPLC H-Class System during the lab-to-lab study was excellent, which is the final key to successful method transferability between sites.

References

1. USP Monograph, Ziprasidone HCl, USP35-NF29, The United States Pharmacopeia Convention, official May 1, 2012.
2. USP General Chapter, <621> Chromatography, USP35-NF30, The United States Pharmacopeia Convention, official December 1, 2012.
3. Jones MD, Alden P, Fountain KJ, Aubin A. Implementation of Methods Translation between Liquid Chromatography Instrumentation. Waters Application Note 720003721en. 2010 Sept.

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