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Application Note

Transfer of Two USP Compendial Methods for Impurities of Ziprasidone HCl to a Single UPLC Method

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Abstract

In this study, we combine two compendial methods into one UPLC method for the separation of ziprasidone HCl impurities to reduce analysis time and improve chromatographic separation.

Benefits

- Method transferability between different systems and different sites
- Faster assay run times
- Savings in mobile phase consumption
- Reduced cost for solvent and waste disposal

Introduction

The majority of today's compendial methods are considered outdated by both industry and regulators, and do not take advantage of the recent advances in recent chromatographic instrumentation and stationary phase solutions. The U.S. Pharmacopeia (USP) launched an initiative in May 2010 to modernize monographs for small molecule drug products and excipients, identified as a priority by the U.S. Food and Drug Administration, that use outdated technology, have safety or environmental concerns, or are missing key aspects.

HPLC methods listed in the United States Pharmacopeia (USP) National Formulary (NF) monographs are typically long and consume a high volume of solvents. With the evolution of new technology, many companies want to migrate to UPLC technology with sub-2-µm particle columns to reduce analysis time and solvent usage, improve chromatographic performance, and maintain quality. Adopting UPLC technology streamlines laboratory processes by improving efficiency, productivity, and profitability of pharmaceutical manufacturing facilities.

Ziprasidone HCl is an anti-psychotic drug administered orally to treat acute manic or mixed episodes associated with bipolar disorder. The USP specifies two HPLC methods[1] to analyze impurities of ziprasidone HCl. In this study, we combine two compendial methods into one UPLC method for the separation of ziprasidone HCl impurities to reduce analysis time and improve chromatographic separation. Performance of

the UPLC method is measured by evaluating five replicate injections of the system suitability solution against the requirements listed in the USP monograph for ziprasidone HCl.

Experimental

Sample description

All solutions, shown in Table 1, were prepared in diluent (methanol/water/HCl at 20:5:0.01) as per the impurities methods defined in the USP monograph for ziprasidone HCl.[1] Since the USP does not list a sample preparation protocol for the Ziprasidone HCl capsules, the drug product 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
Standard solutions	**System suitability solution:** 0.24 mg/mL of ziprasidone HCl 0.5 µg/mL of related compound A 0.8 µg/mL of related compound B	**System suitability solution:** 0.24 mg/mL of ziprasidone HCl 0.8 µg/mL of related compound C 0.8 µg/mL of related compound D
	Standard solution: 0.5 µg/mL of related compound A 0.8 µg/mL of related compound B	**Standard solution:** 0.8 µg/mL of related compound C 0.8 µg/mL of related compound D
Sample solutions	**Sample solution:** 0.4 mg/mL of capsule content	**Sample solution:** 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

Empower 3 Software

Method conditions

HPLC conditions for late-eluting impurities method

LC system:	Alliance 2695 HPLC with 2489 UV/Visible 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

USP system suitability criteria for early-eluting impurities method

For five replicate injections of the 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%

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Separation mode:	Isocratic
Wash solvents:	50:50 water/methanol
Detection:	UV, 229 nm

USP system suitability criteria for late-eluting impurities method

For five replicate injections of the system suitability solution:

- Resolution between ziprasidone and ziprasidone related compound C: NLT 6.0
- Relative standard deviation (RSD) for ziprasidone related compound C: NMT 10%

UPLC conditions

LC system:	ACQUITY UPLC H-Class with ACQUITY UPLC 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:	0.5 mL/min
Mobile phase:	Solvent A: 50mM Potassium phosphate monobasic, pH 6.0
Solvent B:	Methanol
Separation mode:	Gradient
Wash solvents:	50:50 water/methanol
Detection:	UV, 229 nm

Gradient

Step	Time (minutes)	%A	%B
1	Initial	65.0	35.0
2	0.60	35.0	65.0
3	2.70	25.0	75.0

Step	Time (minutes)	%A	%B
4	3.90	25.0	75.0
5	3.96	65.0	35.0
6	6.50	65.0	35.0

Results and Discussion

Two compendial methods for the early and late eluting impurities of ziprasidone HCl were analyzed as described using the Alliance 2695 HPLC System equipped with a 2489 UV/Visible Detector. The USP designates an L7 column, specifically a Zorbax RX-C_8 column, for the impurities testing. Using the Waters Reversed-Phase Column Selectivity Chart (www.waters.com/selectivitychart), an equivalent Waters XBridge C_8 Column was chosen. Chromatographic data for both HPLC methods are displayed in Figure 1.

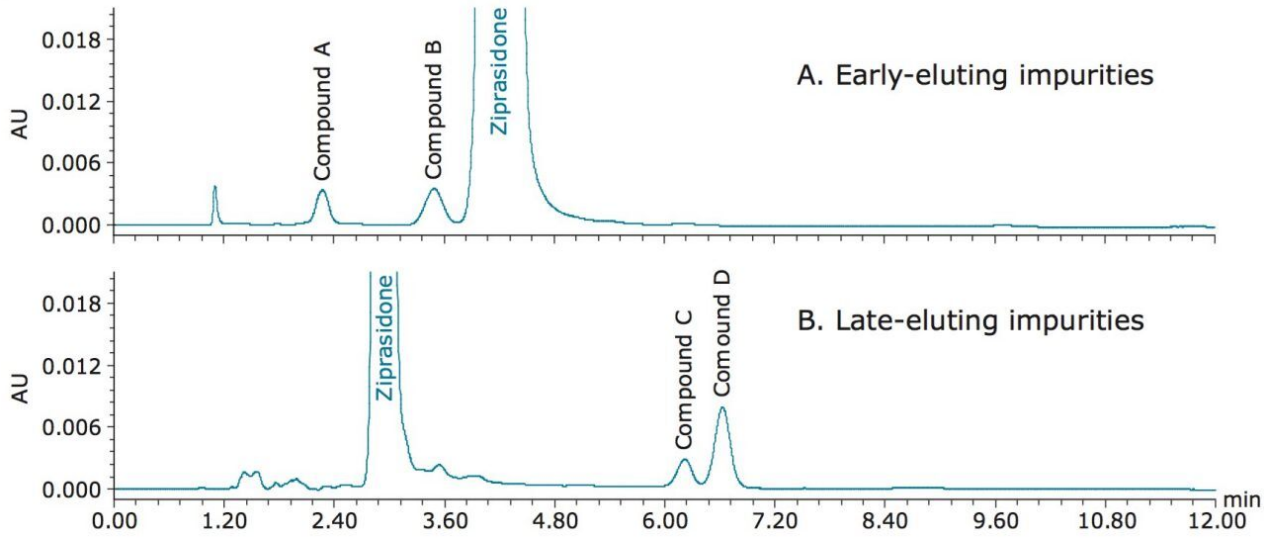

Figure 1. HPLC data of the system suitability solutions acquired on the Alliance HPLC System

A. Early-eluting impurities method

B. Late-eluting impurities method

UPLC method development

Method development of a UPLC-based separation of ziprasidone HCl and all the specified related compounds was conducted by a gradient elution using a potassium phosphate monobasic buffer with pH of 6.0 and methanol. The mobile phase with a neutral pH 6.0, specified in the late-eluting impurities method, was investigated due to the neutral characteristics of the ziprasidone HCl related substances.

A Waters ACQUITY UPLC BEH C_8 Column has the same stationary phase as the Waters HPLC XBridge C_8 Column, hence it was selected for method development. The dimensions of the UPLC Column were decided by the column length (L) to the particle size (dp) ratio (L/dp). The L/dp value for the HPLC column is 30,000. The L/dp value for the UPLC Column with a dimension of 50 mm in length and 1.7-µm particle size is 29,412. Injection volume was scaled down to UPLC using the Waters ACQUITY UPLC Columns Calculator, as previously described.[2]

A generic scouting gradient from 5% to 95% methanol over 15 minutes was performed to investigate the elution and separation between the peaks. Increasing the starting percent of the organic solvent to 35% lowered the run time, providing an adequate separation between all the peaks. Examples of the chromatographic data for the gradient elution study are displayed in Figure 2.

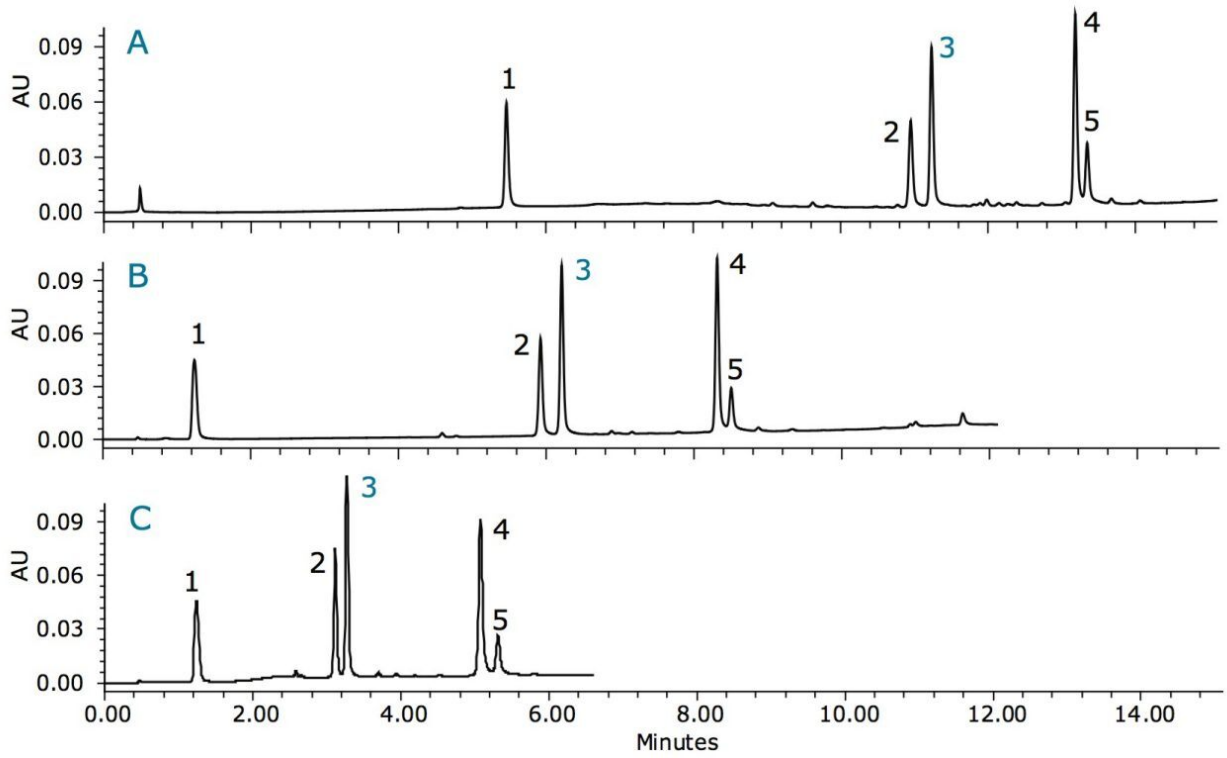

Figure 2. Gradient elution study for UPLC method development.

Peak 1: Related compound A

Peak 2: Related compound B

Peak 3: Ziprasidone

Peak 4: Related compound D

Peak 5: Related compound C

A. 5% to 95% methanol (Solvent B) over 15 minutes

B. 35% to 65%B over 5.0 minutes, 65% to 90%B over 5.0 minutes, hold at 90% for 2.0 minutes

C. 35% to 65%B over 1.0 minute, 65% to 75%B over 3.5 minutes, hold at 75% for 2.0 minutes

Different flow rates were also explored to further reduce run time and determine the effect on resolution. The flow rate was increased from 0.3 to 0.5 mL/min, as shown in Figure 3. It was observed that an increase in flow rate lowered the resolution between peaks 2 (related compound B) and 3 (ziprasidone), but still passing the USP criteria for the system suitability, shown in Table 2. In addition, a flow rate of 0.5 mL/min reduced run time from 6.6 to 4.0 minutes and decreased peak width by 32%.

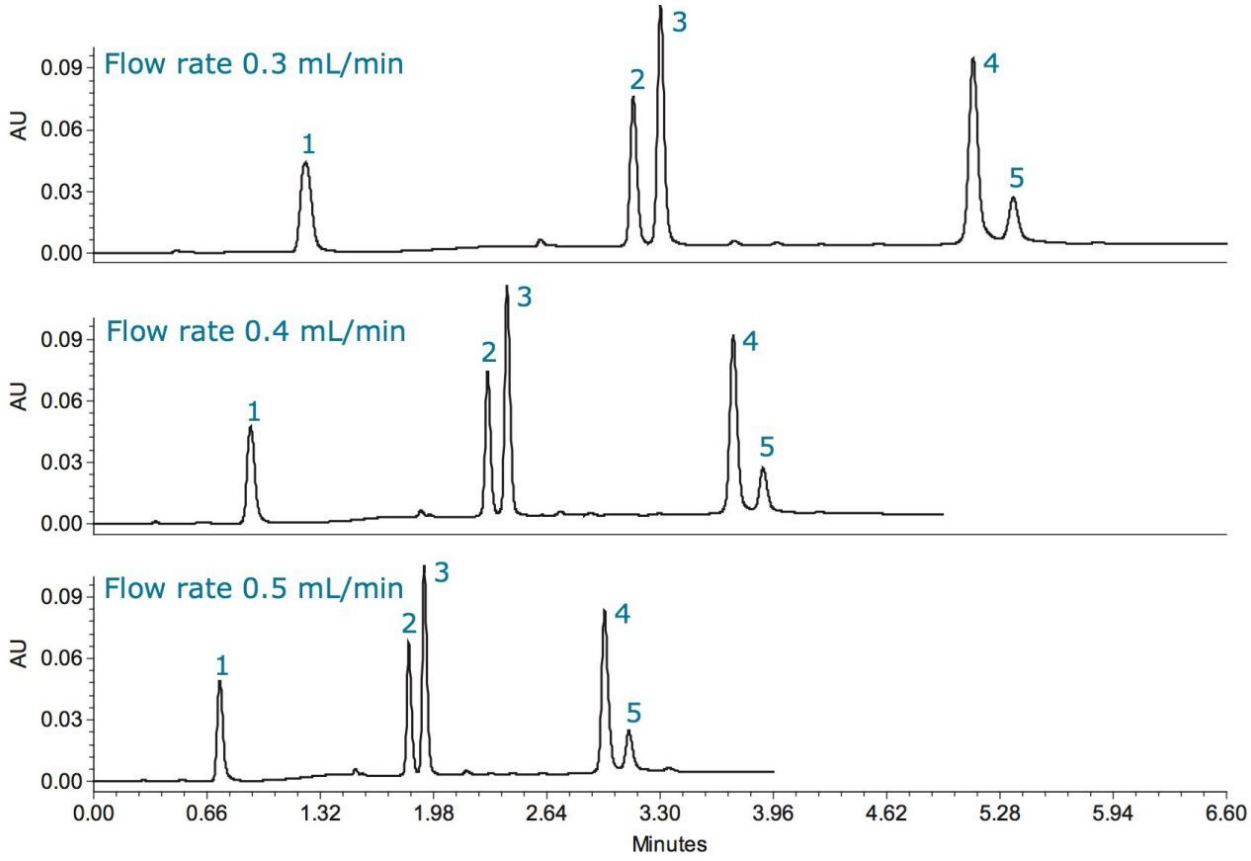

Figure 3. Flow rate study for UPLC method development.

Peak 1: Related compound A

Peak 2: Related compound B

Peak 3: Ziprasidone HCl

Peak 4: Related compound D

Peak 5: Related compound C

The resulting UPLC method successfully resolved ziprasidone and all the specified related compounds, shown in Figure 4.

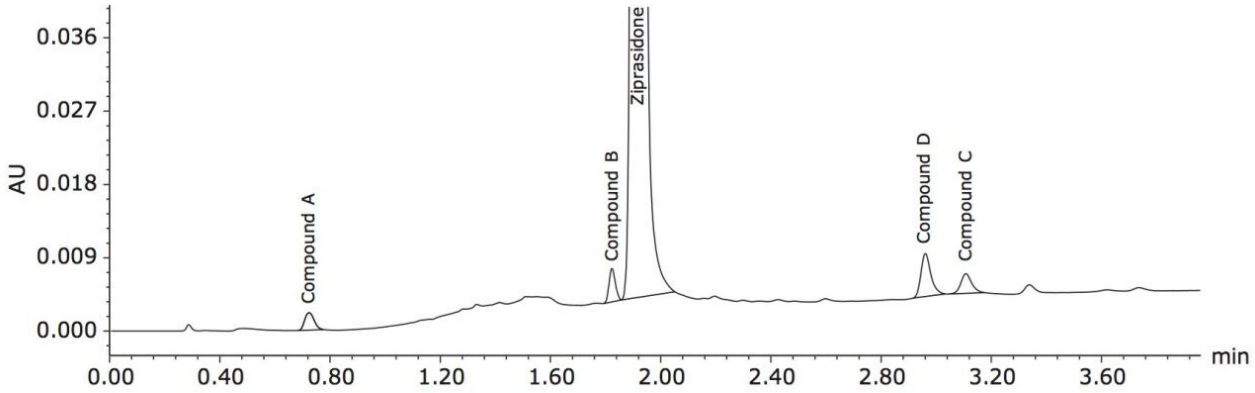

Figure 4. Final UPLC method. System suitability solution was injected onto ACQUITY UPLC BEH C_8 , 2.1 x 5 0 mm, 1.7 µm Column with an amount of 1.4 µL. Column temperature was maintained at 35 °C. Mobile phase consisting solvent A: 50 mM potassium phosphate monobasic with pH of 6.0 and solvent B: methanol was delivered via a gradient elution with a flow rate of 0.5 mL/min.

Performance of the UPLC method was determined by comparing the system suitability results of the five replicate injections of the system suitability solutions against the acceptance criteria defined in the USP monograph for ziprasidone HCl. System suitability results met the USP requirements for the early and late impurities methods listed in the USP monograph for ziprasidone HCl, shown in Table 2.

System suitability parameters	USP criteria	UPLC results
Early-eluting impurities method		
• Resolution between ziprasidone and related compound B	• NLT 1.5	• 2.02
• %RSD for related compound B	• NMT 10%	• RT: 0.1% • Area: 0.2%
Late-eluting impurities method		
• Resolution between ziprasidone and related compound C	• NLT 6.0	• 19.1*
• %RSD for related compound C	• NMT 10%	• RT: 0.1% • Area: 0.2%

Table 2. System suitability results for five replicate injections of the system suitability solution on an ACQUITY UPLC H-Class System.

**Note: Elution order for related compounds C and D has changed with analysis on UPLC. Value reported in the table reflects resolution between ziprasidone and related compound D*

Conclusion

- UPLC methodology successfully separated ziprasidone HCl and all related compounds indicated by the USP monograph for ziprasidone HCl.
- The UPLC method provides a 75% reduction in run time compared to the two compendial procedures, while meeting the USP criteria for system suitability.
- The amount of mobile phase per UPLC injection is 3.25 mL compared to 30.00 mL for the two compendial HPLC methodologies, which represents an 89% savings in mobile phase consumption.
- Implementing UPLC technology provides improvements in laboratory throughput and productivity by reducing analysis time for release testing of manufacturing batches.

References

1. USP Monograph, Ziprasidone HCl, USP35-NF29, The United States Pharmacopeia Convention, official May 1, 2012.
2. 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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