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Modernizing Chromatographic Methods in Alignment with USP <621> General Chapter

Jonathan E. Turner

Principal Product Marketing Manager

Waters Corporation

Organized by ISPE Boston Area Chapter

March 24th, 2022

Agenda

- Overview of USP and Chapter <621>
- Current and Approved Updates <621>
- Case Study
  - Isocratic and Gradient USP Method Modernization Using Approved Updates

What is the USP?

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- USP is an **independent, scientific nonprofit organization** focused on building trust in the supply of safe, quality medicines.

- USP is an **official quality standard for medicines marketed in the US**. In addition, USP is utilized in over 140 countries worldwide and integrated into the laws of more than 40 countries.

- The United States Pharmacopeia – National Formulary (USP-NF) includes over 5000 quality standards for medicines, both chemical and biologic; active pharmaceutical ingredients (APIs); and excipients (inactive ingredients).

- **USP has no role in enforcing its standards**; enforcement is the responsibility of Food and Drug Association (FDA) and other government authorities in the U.S. and elsewhere.

USP General Chapter *Chromatography* <621>

- USP-NF <621> are the guidelines governing allowed adjustments to chromatographic systems.
  - It “defines the terms and procedures used in chromatography and provides general information regarding system suitability

- USP-NF <621> is periodically updated to reflect/incorporate industry changes when it comes to new LC systems and columns

- The Pharmacopeial Discussion Group (PDG) of the European Pharmacopoeia, the Japanese Pharmacopoeia, and the United States Pharmacopeia has expressed a **commitment to achieving harmonization of <621>**

Method Validation, Verification and Transfer
Where <621> Fits

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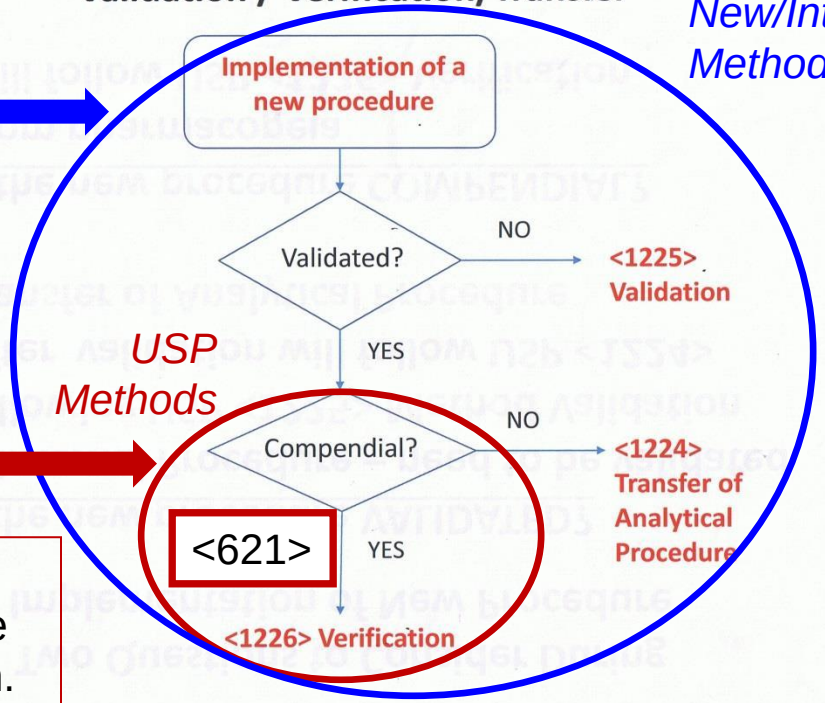
Two Questions to Consider During Implementation of New Procedure

1. **Is the new procedure VALIDATED?**
   - If Internal Procedure – need to be validated following USP <1225> Method Validation
   - After validation will follow USP <1224> Transfer of Analytical Procedure

2. **Is the new procedure COMPENDIAL?**
   - From pharmacopeia
   - Will follow USP <1226> Verification

Validation / Verification/Transfer

New/Internal Methods



Compendial methods **must pass the system suitability requirement(s)**, and these need to be verified with the API and/or Final drug formulation.

USP Chapter <621> Chromatography Defines “Allowable Adjustments”

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“Adjustments to the specified chromatographic system may be necessary in order to meet system suitability requirements.”

- Allowable adjustments permitted only when:
  - Suitable standards are available for all compounds used in suitability test
  - Adjustments (or column change) yields results that meet all system suitability requirements specified in official procedure.
- Must use the same L-designation of column

USP “L” Column Listing

L1	Octadecyl silane (ODS or C_{18}) chemically bonded to porous silica or ceramic particles - 1.5 to 10 µm in diameter. See new subclassification table on previous page.

USP ⟨621⟩ Guidelines (as of Aug 1, 2017) (USP43-NF38)

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Variable	Isocratic	Gradient
Stationary phase	No change of the physio-chemical characteristics of the stationary phase (Same L category)	
Particle size/ Column length	Per constant L/d_p or N: -25% to +50%	No Changes Allowed
Column diameter	Can be adjusted if the linear velocity is kept constant	No Changes Allowed
Flow rate	Based on particle size and ± 50%	No Changes Allowed
Injection volume	Flexible	
Column temperature	± 10ºC	
Mobile phase pH	± 0.2 pH units	
Mobile phase composition	± 30 % relative (minor component), ± 10 % absolute	

What's new in <621>
Coming December 1st 2022

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- **Distinction between** total porous (**TPP**) and superficially porous (**SPP**) particles
  - Provision when replacing TPP with SPP
  - Requires **accounting for efficiency gain** when SPPs are used in lieu of TPP
- **Column size adjustments** now **allowed for both isocratic and gradient methods**
  - **Column length and particle size** affecting efficiency now based on **L/dp ratio**
  - **Column diameter** can be adjusted with a warning for impact of extra-column band broadening factors added
- **Gradient time adjustment permitted** when changing column dimension
- **Injection volume can adjusted**. Introduced relationship with column length and i.d.
- **pH and Temperature in gradient mode** can be adjusted – same ranges as isocratic conditions

Roadblocks towards modern chromatography removed

USP ⟨621⟩ Guidelines (as of Dec 1, 2022)

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Variable	Isocratic	Gradient
Stationary phase	No change of the physio-chemical characteristics of the stationary phase (Same USP L category as referenced in the monograph)	
Particle size/ Column length	**L/d_p -25% to +50%*** **From TPP to SSP $(t_R/w_h)^2$ for gradient or N for isocratic -25% to +50%**	
Column diameter	**May be adjusted***	
Flow rate	**Scaled according to new d_p and d_c^2**	
	Additional ± 50% permitted	After flow scaling, adapt t_g to t_0
Injection volume	Flexible, provided SST criteria are met	
Column temperature	± 10ºC	± 5ºC
Mobile phase pH	pH : ± 0.2 pH units	
Mobile phase composition	± 30 % relative (minor component), ± 10 % absolute	Adjustments acceptable if: ❶ SST criteria fulfilled, ❷ Rt's ± 15% original, ❸ Retention for early eluters / elution for late peaks achieved

(*) **verify instrument ECV impact**

Isocratic Case Study:
USP Quetiapine Fumarate Assay

HPLC UHPLC UPLC

Quetiapine Fumarate **Assay** USP 40 NF35 S1 Column Selection

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ASSAY

- **PROCEDURE**

Buffer: 2.6 g/L of dibasic ammonium phosphate. Adjust with phosphoric acid to a pH of 6.5.

Mobile phase: Methanol, acetonitrile, and *Buffer* (54:7:39)

Mode: LC

Detector: UV 230 nm

Column: 4.6-mm × 25-cm; 5-µm packing L7

Flow rate: 1.3 mL/min

Injection volume: 50 µL

L7	Octyl silane (C_8) chemically bonded to porous silica particles - 1.5 to 10 µm in diameter.

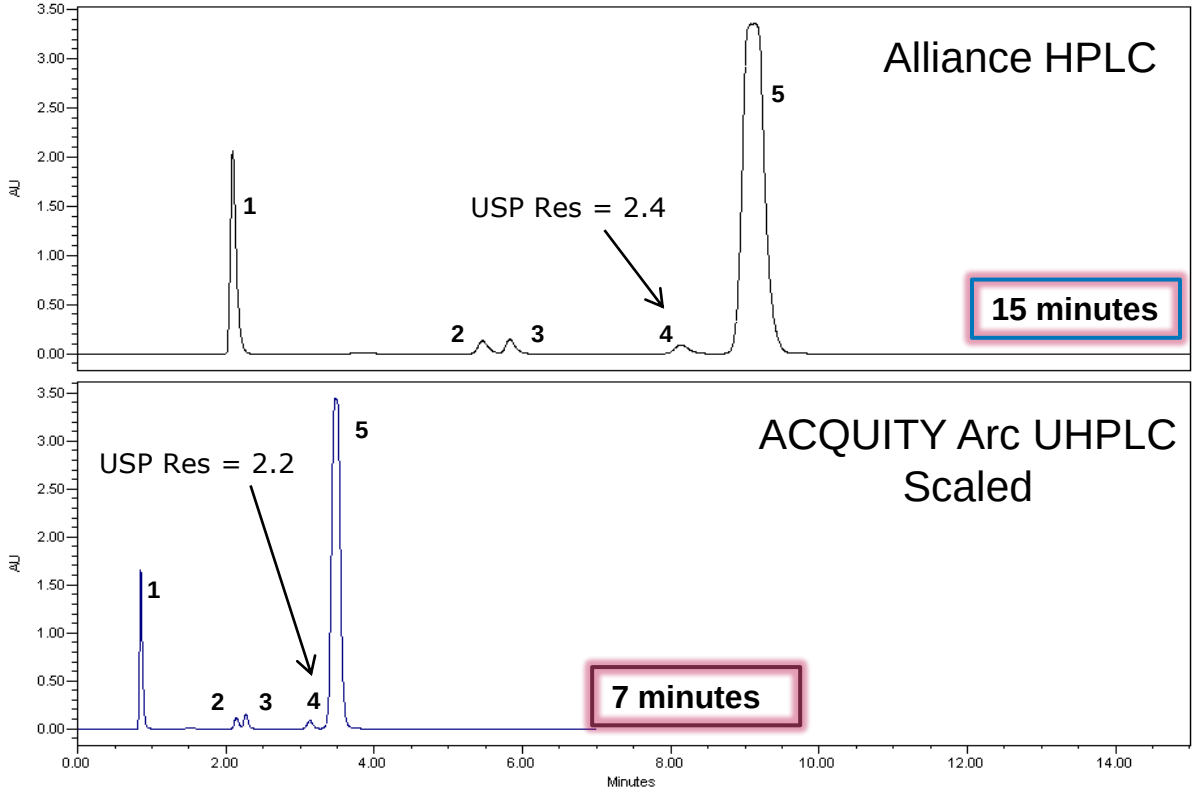
HPLC Column:
XBridge BEH C8, **5 µm**,
4.6 mm x 250 mm

UHPLC Column:
XBridge BEH C8, **3.5 µm**,
3.0 mm x 150 mm

Allowed L/dp:
-25 % to +50 %

Sample Temp: 4°C
Column Temp: 25°C
Needle Wash: 70:30 Methanol:Water

Isocratic Scaling Results
Alliance HPLC to ACQUITY Arc UHPLC



- Inj Vol: 50.0 µL
- Flow Rate: 1.3 mL/min
- Run Time: 15 minutes
- Column: XBridge BEH C8, 5 µm, 4.6 mm x 250 mm
- L/dp: 50,000

- Inj Vol: 12.8 µL
- Flow Rate: 0.800 mL/min
- Run Time: 7 minutes
- Column: XBridge BEH C8, 3.5 µm, 3.0 mm x 150 mm
- L/dp: 42,857; -14% ✓

Scaling USP Quetiapine Fumarate Assay Results Alliance HPLC to ACQUITY Arc UHPLC

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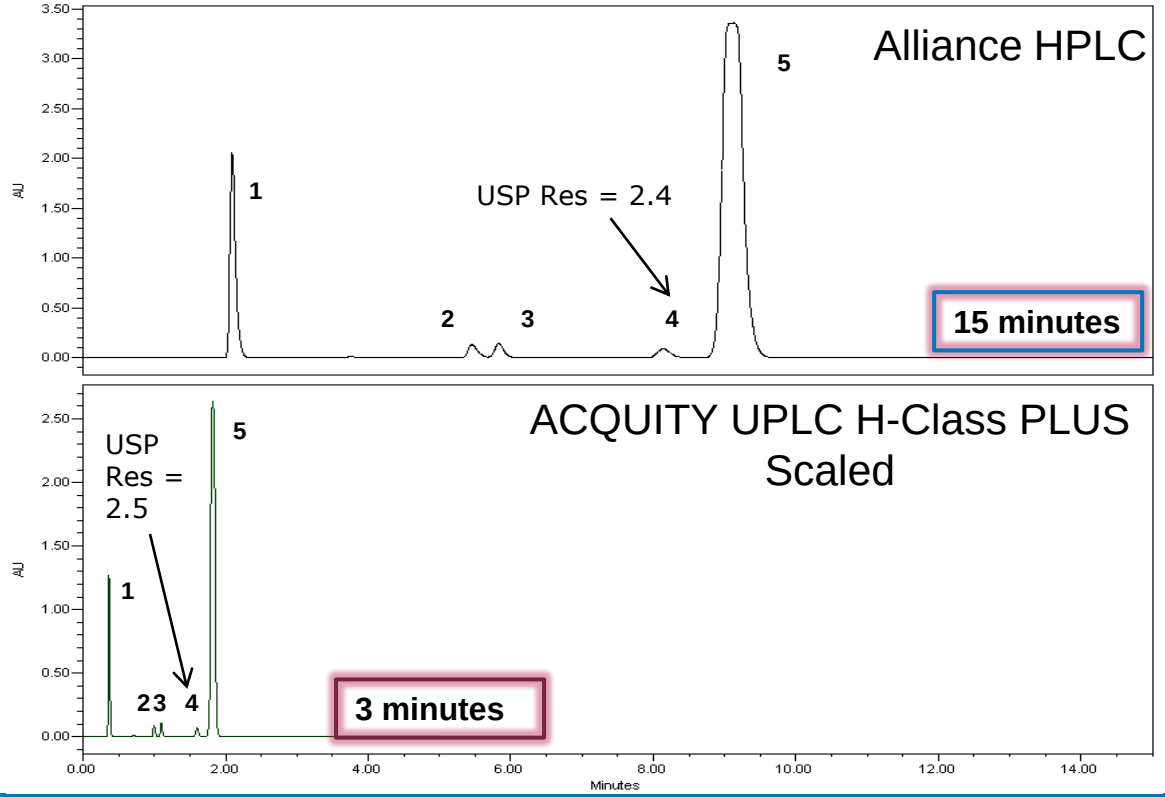
	Resolution (peak 4 &5)	Quetiapine Tailing	Quetiapine Area %RSD	Quetiapine Retention Time %RSD	Unknown Sample Result	Run Time (minutes)	Solvent Consumption per Sample (mL)
Alliance HPLC	2.4 ✓	1.2 ✓	0.09 ✓	0.16 ✓	109.5	15	19.5
ACQUITY Arc UHPLC	2.2 ✓	1.1 ✓	0.09 ✓	0.02 ✓	108.9	7	5.6
USP Requirements	NLT 1.5 (Syst. Suit.)	NMT 2.0 (Standard)	NMT 2.0% (Standard)	NMT 2.0% (Standard)	---	---	---

from 4 to 8.6 samples/hour

from 19.5 to 5.6 mL/sample

Isocratic Scaling Results
Alliance HPLC to ACQUITY UPLC H-Class PLUS



- Inj Vol: 50.0 µL
- Flow Rate: 1.3 mL/min
- Run Time: 15 minutes
- Column: XBridge BEH C8, 5 µm, 4.6 mm x 250 mm
- L/dp: 50,000

- Inj Vol: 3.1 µL
- Flow Rate: 0.600 mL/min
- Run Time: 3 minutes
- Column: ACQUITY UPLC BEH C8, 1.7 µm, 2.1 mm x 75 mm
- L/dp: 44,118, -12% ✓

Scaling USP Quetiapine Fumarate Assay Results Alliance HPLC to ACQUITY UPLC H-Class PLUS

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	Resolution (peak 4 &5)	Quetiapine Tailing	Quetiapine Area %RSD	Quetiapine Retention Time %RSD	Unknown Sample Result	Run Time (minutes)	Solvent Consumption per Sample (mL)
Alliance HPLC	2.4 ✓	1.2 ✓	0.09 ✓	0.16 ✓	109.5	15	19.5
ACQUITY UPLC H-Class PLUS	2.5 ✓	1.1 ✓	0.01 ✓	0.10 ✓	109.7	3	2.4
USP Requirements	NLT 1.5 (Syst. Suit)	NMT 2.0 (Standard)	NMT 2.0% (Standard)	NMT 2.0% (Standard)	---	---	---

from 4 to 20 samples/hour

from 19.5 to 2.4 mL/sample

Scaling USP Quetiapine Fumarate Assay Results

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	Resolution (peak 4 & 5)	Quetiapine				
		Tailing	Area %RSD	R_t %RSD	Samples per hour	Solvent Consumption per Sample (mL)
HPLC Alliance	2.4	1.2	0.09	0.16	4	19.5
UHPLC ACQUITY Arc	2.2	1.1	0.09	0.02	8.6	5.6
UPLC ACQUITY H-Class PLUS	2.5	1.1	0.01	0.10	20	2.4
USP Requirements	NLT 1.5 (Syst. Suit)	NMT 2.0 (Standard)	NMT 2.0% (Standard)	NMT 2.0% (Standard)		

Gradient Case Study:
Quetiapine Fumarate Impurities

HPLC UHPLC UPLC

Quetiapine Fumarate **Impurities Method** USP 40 NF35 S Column Selection

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- **Organic Impurities**

Buffer: 3.1 g/L of ammonium acetate in water. Add 2 mL of 25% ammonium hydroxide to each 1 L of solution. The pH of the resulting solution is NLT 9.2.

Solution A: Acetonitrile and *Buffer* (25:75)

Solution B: Acetonitrile

Diluent: *Solution A* and *Solution B* (86:14)

Mode: LC

Detector: UV 250 nm

Column: 4.6-mm × 15-cm; 3.5-µm packing L7

Column temperature: 45°

Flow rate: 1.5 mL/min

Injection volume: 20 µL

Time (min)	Solution A	Solution B
0.0	100	0.0
25.0	100	0.0
60.0	29.3	70.7
60.1	100	0.0
68.0	100	0.0
70.0	100	0.0

HPLC

XBridge BEH C8
3.5 µm,
4.6 mm x 150 mm
L/dp: 42,857

→

UHPLC

XBridge BEH C8
2.5 µm,
3.0 mm x 100 mm
L/dp: 40,000

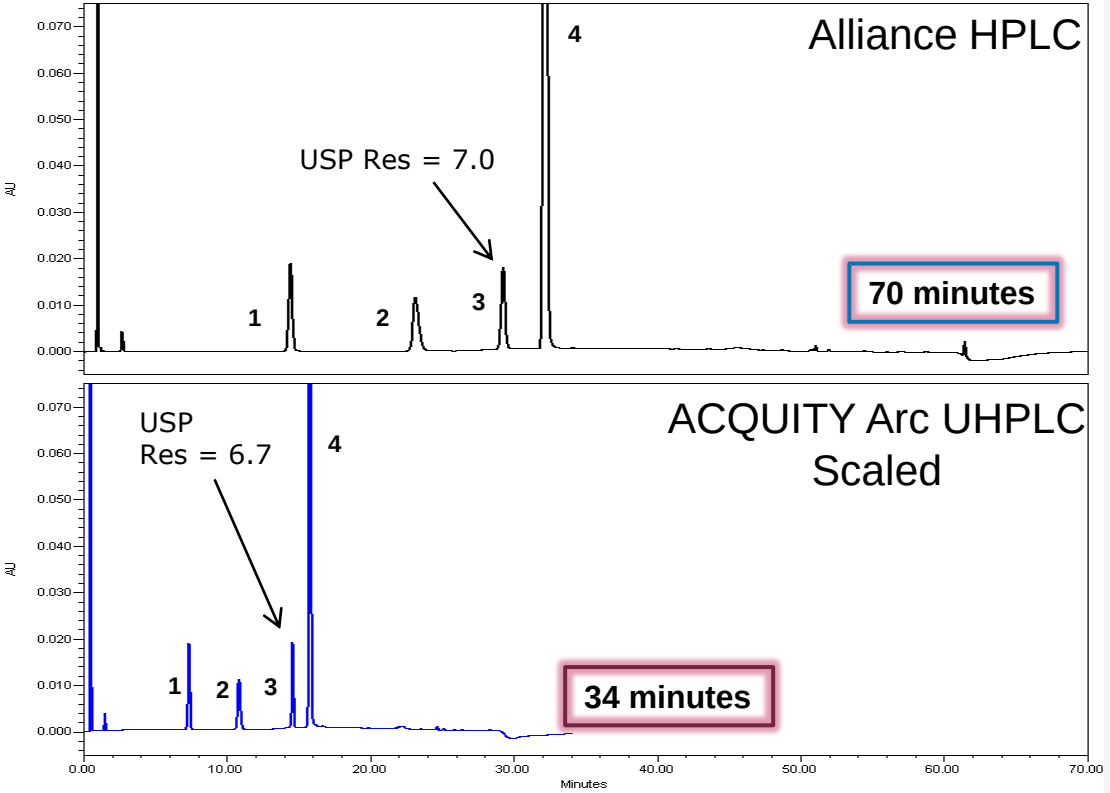
→

UPLC

ACQUITY UPLC BEH C8
1.7 µm,
2.1 mm x 75 mm
L/dp: 44,118

Allowed L/dp:
-25 % to +50 %

Gradient Scaling Results
Alliance HPLC to ACQUITY Arc UHPLC



UHPLC Scaled Gradient:

Time (min)	Solution A	Solution B
0.0	100	0.0
11.90	100	0.0
28.57	29.3	70.7
28.62	100	0.0
32.38	100	0.0
34.00	100	0.0

- Inj Vol: 5.7 µL
- Flow Rate: 0.893 mL/min
- Run Time: 34 minutes
- Column: XBridge BEH C8, 2.5 µm, 3.0 mm X 100 mm
- Pre-Injection Volume: 388 µL
- L/dp: 40,000, -6% ✓

Scaling USP Quetiapine Fumarate Impurities Method Results Alliance HPLC to ACQUITY Arc UHPLC

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	Resolution (peak 1 & 2)	Resolution (peak 4 & 5)	Quetiapine Tailing	Quetiapine Area %RSD	Quetiapine Retention Time %RSD	Run Time (minutes)	Solvent Consumption per Sample (mL)
Alliance HPLC	14.0 ✓	7.0 ✓	1.03 ✓	1.24 ✓	0.04	70	105
ACQUITY Arc UHPLC	13.2 ✓	6.7 ✓	0.95 ✓	0.57 ✓	0.02	34	30
USP Requirements	NLT 3.0 (Syst. Suit)	NLT 4.0 (Syst. Suit)	NMT 2.0 (Standard)	NMT 2.0% (Standard)	NMT 2.0% (Standard)	---	---

from 0.9 to 1.8 samples/hour

from 105 to 30 mL/samples

Unknown Sample	Quetiapine desethoxy	Unknown Impurity	Total Impurities
Alliance HPLC	0.12%	0.08%	0.22%
ACQUITY Arc UHPLC	0.09%	0.06%	0.17%
USP Acceptance Criteria*	NMT 0.15%	NMT 0.10%	NMT 0.50%

*Disregard peaks below 0.05% or with retention times less than 2 minutes

Quetiapine Fumarate **Impurities Method** USP 40 NF35 S1 Alliance HPLC to ACQUITY UPLC H-Class PLUS

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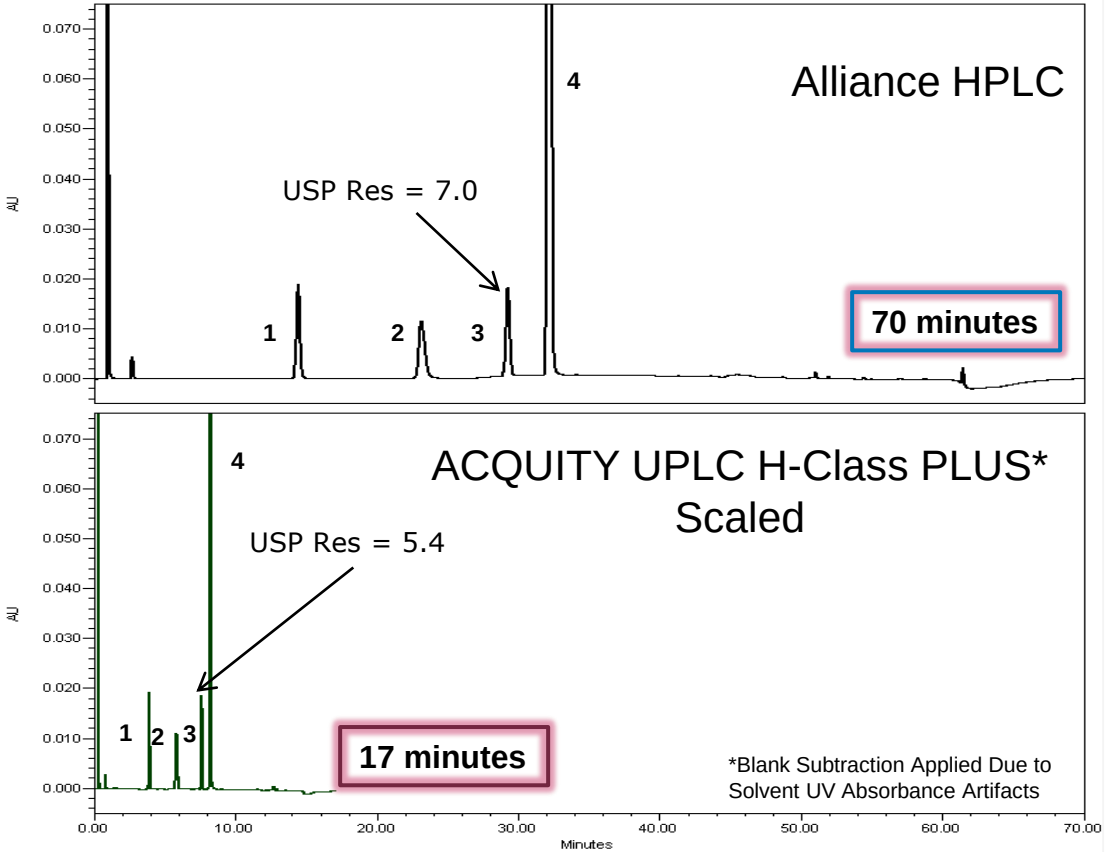

UPLC Scaled Gradient:

Time (min)	Solution A	Solution B
0.0	100	0.0
6.07	100	0.0
14.57	29.3	70.7
14.60	100	0.0
16.51	100	0.0
17.00	100	0.0

- Inj Vol: 2.1 µL
- Flow Rate: 0.644 mL/min
- Run Time: 17 minutes
- Column: ACQUITY UPLC BEH C8, 1.7 µm, 2.1 mm X 75 mm
- Pre-Injection Volume: 285 µL
- L/dp: 44,118, -3% ✓

Scaling USP Quetiapine Fumarate Impurities Method Results Alliance HPLC to ACQUITY UPLC H-Class PLUS

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	Resolution (peak 1 & 2)	Resolution (peak 4 & 5)	Quetiapine Tailing	Quetiapine Area %RSD	Quetiapine Retention Time %RSD	Run Time (minutes)	Solvent Consumption per Sample (mL)
Alliance HPLC	14.0 ✓	7.0 ✓	1.03 ✓	1.24 ✓	0.04	70	105
ACQUITY UPLC H-Class PLUS	11.2 ✓	5.4 ✓	1.04 ✓	0.65 ✓	0.02	17	11
USP Requirements	NLT 3.0 (Syst. Suit)	NLT 4.0 (Syst. Suit)	NMT 2.0 (Standard)	NMT 2.0% (Standard)	NMT 2.0% (Standard)	---	---

from 0.9 to 3.5 samples/hour

from 105 to 11 mL/samples

Unknown Sample	Quetiapine desethoxy	Unknown Impurity	Total Impurities
Alliance HPLC	0.12%	0.08%	0.22%
ACQUITY UPLC H-Class PLUS	0.10%	0.07%	0.19%
USP Acceptance Criteria*	NMT 0.15%	NMT 0.10%	NMT 0.50%

*Disregard peaks below 0.05% or with retention times less than 2 minutes

Scaling USP Quetiapine Fumarate Impurities Method Results

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	Resolution (peak 1 & 2)	Resolution (peak 4 & 5)	Quetiapine Tailing	Quetiapine Area %RSD	Quetiapine Retention Time %RSD	Run Time (minutes)	Solvent Consumption per Sample (mL)
Alliance HPLC	14.0	7.0	1.03	1.24	0.04	70	105
ACQUITY Arc UHPLC	13.2	6.7	0.95	0.57	0.02	34	30
ACQUITY UPLC H-Class PLUS	11.2	5.4	1.04	0.65	0.02	17	11
USP Requirements	NLT 3.0 (Syst. Suit)	NLT 4.0 (Syst. Suit)	NMT 2.0 (Standard)	NMT 2.0% (Standard)	NMT 2.0% (Standard)	---	---

Summary

- USP Chapter <621> provides the chromatography guidelines for allowable method changes
  - These can be used to modernize existing methods
  - This is for both compendial and inhouse created methods

- Current <621> guidelines allow changes in particle size, column configuration, temperature, and flow rate are allowed

- Updates to Chapter <621> will provide guidelines for allowable changes to gradient methods
  - Expected time frame for approval is December 1, 2022

Thank You for Your Time

USP-NF FAQs: <621> Chromatography

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- **1. To what degree can a chromatographic procedure be modified and still be in compliance? Can column length, internal diameter, mobile phase composition be modified?**
  - Chromatography General Chapter <621> contains a list of allowed adjustments to chromatographic systems. However, the user should verify the suitability of the method under the new conditions by assessing the relevant analytical performance characteristics potentially affected by the change.
- **2. What brand of HPLC/GC column was used in the development and/or validation of a particular test? Is there an alternative chromatographic column for a particular test?**
  - The most updated information on the brand name of the column used to validate any chromatographic procedure in USP—NF, together with possible alternatives, where applicable are available at the following www.uspchromcolumns.com.
- **3. How much deviation is allowed from a relative retention time prescribed in a monograph**?
  - From <621>, the deviations of relative retention time values measured for the test substance from the values obtained for the reference compound and mixture should not exceed the reliability estimates determined statistically from replicate assays of the reference compound. Also, relative retention times may be provided in monographs for informational purposes only, to aid in peak identification. There are no acceptance criteria applied to relative retention times.

[1]http://www.usp.org/frequently-asked-questions/chromatography