Application Note

Pharma & Biopharma

Agilent

Trusted Answers

Reduced Solvent Use and Analysis Time According to USP Methods

Method transfer to UHPLC conditions according to USP Chapter <621> requirements, using the Agilent 1260 Infinity II Prime LC System

Suitable for Agilent
1260 Infinity III LC

Authors

Edgar Naegele and
Sonja Schipperges
Agilent Technologies, Inc.

Abstract

This application note demonstrates gradient separation of a pharmaceutical compound from its impurities under standard conditions, according to United States Pharmacopeia (USP) requirements. Gradient separation is improved by method transfer to UHPLC conditions for smaller column i.d. and smaller particle size, while retaining the separation requirements listed in the respective USP method and the calculations given in USP <621>. This is demonstrated using the Agilent 1260 Infinity II Prime LC, which is capable of working up to 800 bar. In the first place, method transfer to UHPLC is beneficial for shorter analysis time with retained separation performance and reduction of analysis time and costs. As a result, this method transfer also helps you achieve your lab's sustainability goals and realize cost savings in quality control (QC).

This application note also features an interactive cost savings calculator. Use this tool to see how the Agilent 1260 Infinity II Prime LC System can help to save resources, translating into cost and solvent savings for your specific analysis.

Note: This PDF contains interactive tables. You can modify values to match your requirements. The calculation in the table will update accordingly. To keep your values, please download and save the document. Please note that modification of table values won't be reflected in the text.

Introduction

The USP provides general guidelines related to the tests and procedures described in monographs. Chapter <621> provides specialized guidelines on chromatography.[1] Changes recently made to USP chapter <621> now permit adjustments of chromatographic gradient conditions in liquid chromatography. With this revision of this chapter, changes in column dimensions, as well as the transfer from totally porous particle (TPP) columns to superficially porous particle (SPP) columns, are acceptable for gradient elution in liquid chromatography. Table 1 provides an overview of the permitted adjustments of chromatographic conditions for gradient systems.

This application note describes the method transfer from a USP monograph starting at standard HPLC conditions, to UHPLC conditions under the requirements according to USP <621>. These are provided for gradient elution liquid chromatography for quetiapine and its organic impurities. The original USP method is applied using a legacy Agilent 1100 Series LC equipped with a TPP column, and is transferred to the Agilent 1260 Infinity II Prime LC in combination with an SPP column. Analysis time, solvent consumption, and resulting cost per injection are compared for the original method and the analysis using UHPLC conditions.

Table 1. Adjustments of chromatographic conditions for gradient systems according to USP chapter <621>.

Parameter	Permitted Adjustments
Column Length and Particle Size	Particle size and/or column length may be modified, provided that the ratio of column length (L) to particle size (dp) remains constant (or between –25% and +50% of the prescribed L/dp ratio). System suitability criteria need to be fulfilled.
Column Internal Diameter	The internal diameter of the column may be adjusted.
Flow Rate	Flow rate is adjusted for the change in column diameter and particle size using the following equation: $F_2 = F_1 \times [(dc_2^2 \times dp_1)/(dc_1^2 \times dp_2)]$
Injection Volume	When the column dimensions are changed, the following equation may be used for adjusting the injection volume: $V_{inj2} = V_{inj1} \times [(L_2 \times dc_2^2)/(L_1 \times dc_1^2)]$
Gradient Times	The new gradient times are calculated from the original gradient times as follows: $t_{G2} = t_{G1} \times (F_1/F_2) \times [(L_2 \times dc_2^2)/(L_1 \times dc_1^2)]$
Column Temperature	±5 °C, where the operating temperature is specified, unless otherwise prescribed.

F_1 = Flow rate indicated in the monograph
F_2 = Adjusted flow rate
dc_1 = Internal diameter of the column indicated in the monograph
dc_2 = Internal diameter of the column used
dp_1 = Particle size of the column indicated in the monograph
dp_2 = Particle size of the column used
V_{inj1} = Injection volume indicated in the monograph
V_{inj2} = Adjusted injection volume
L_1 = Length of the column indicated in the monograph
L_2 = Length of the column used
t_{G1} = Gradient time indicated in the monograph
t_{G2} = Adjusted gradient time

Experimental

Equipment

The Agilent 1100 Series LC System comprised the following modules:

- Agilent 1100 Series Degasser (G1322A)
- Agilent 1100 Series Quaternary Pump (G1311A)
- Agilent 1100 Series Autosampler (G1313A)
- Agilent 1100 Series Thermostatted Column Compartment (G1316A)
- Agilent 1100 Series Diode Array Detector (G1315B) with standard flow cell, 10 mm (G1315-60022)

The Agilent 1260 Infinity II Prime LC System comprised the following modules:

- Agilent 1260 Infinity II Flexible Pump (G7104C)
- Agilent 1260 Infinity II Vialsampler (G7129C)
- Agilent 1260 Infinity II Multicolumn Thermostat (G7116A)
- Agilent 1260 Infinity II Diode Array Detector HS (G7117C) with Agilent InfinityLab Max-Light cartridge cell 10 mm (G4212-60008)

Software

Agilent OpenLab CDS version 2.6

Columns

- Agilent ZORBAX Eclipse XDB-C8, 4.6 × 150 mm, 3.5 µm (part number 993967-906)
- Agilent InfinityLab Poroshell 120 EC-C8, 4.6 × 100 mm, 2.7 µm (part number 695975-906)
- Agilent InfinityLab Poroshell 120 EC-C8, 2.1 × 100 mm, 2.7 µm (part number 695775-906)

Table 2. Method for analysis of organic impurities of quetiapine as described in the USP monograph.[2]

Parameter	Value
Column	Agilent ZORBAX Eclipse XDB-C8, 4.6 × 150 mm, 3.5 µm
Solvent	Solution A: acetonitrile:buffer (75:25 v/v) Solution B: acetonitrile
Gradient	Time (min) %B 0.00 0.00 25.00 0.00 60.00 77.7 60.01 0.00 68.00 0.00 Stop time: 68 min
Flow Rate	1.50 mL/min
Temperature	45 °C
Detection	250 nm/4 nm, reference 360 nm/100 nm, 10 Hz
Injection	Injection volume: 20.00 µL

Table 3. Method for analysis of organic impurities of quetiapine: transfer to a 4.6 × 100 mm, 2.7 µm column.

Parameter	Value
Column	Agilent InfinityLab Poroshell EC-C8, 4.6 × 100 mm, 2.7 µm
Solvent	Solution A: acetonitrile:buffer (75:25 v/v) Solution B: acetonitrile
Gradient	Time (min) %B 0.00 0.00 12.86 0.00 30.86 70.7 30.91 0.00 34.97 0.00 Stop time: 35 min
Flow Rate	1.94 mL/min
Temperature	45 °C
Detection	250 nm/4 nm, reference 360 nm/100 nm, 20 Hz
Injection	Injection volume: 13.33 µL

Table 4. Method for analysis of organic impurities of quetiapine: transfer to a 2.1 × 100 mm, 2.7 µm column.

Parameter	Value
Column	Agilent InfinityLab Poroshell EC-C8, 2.1 × 100 mm, 2.7 µm
Solvent	Solution A: acetonitrile/buffer (75:25 v/v) Solution B: acetonitrile
Gradient	Time (min) %B 0.00 0.00 12.86 0.00 30.86 70.7 30.91 0.00 34.97 0.00 Stop time: 35 min
Flow Rate	0.41 mL/min
Temperature	45 °C
Detection	250 nm/4 nm, reference 360 nm/100 nm, 20 Hz
Injection	Injection volume: 2.78 µL

Buffer solution

Ammonium acetate (3.1 g/L) in water with 2 mL of 25% ammonium hydroxide per 1 L of solution. The pH of the resulting solution must not be lower than 9.2.

Preparation of solutions for system suitability

USP Quetiapine System Suitability RS (1 mg/mL) in diluent (solution A and solution B (86:14)).

Preparation of standard solution

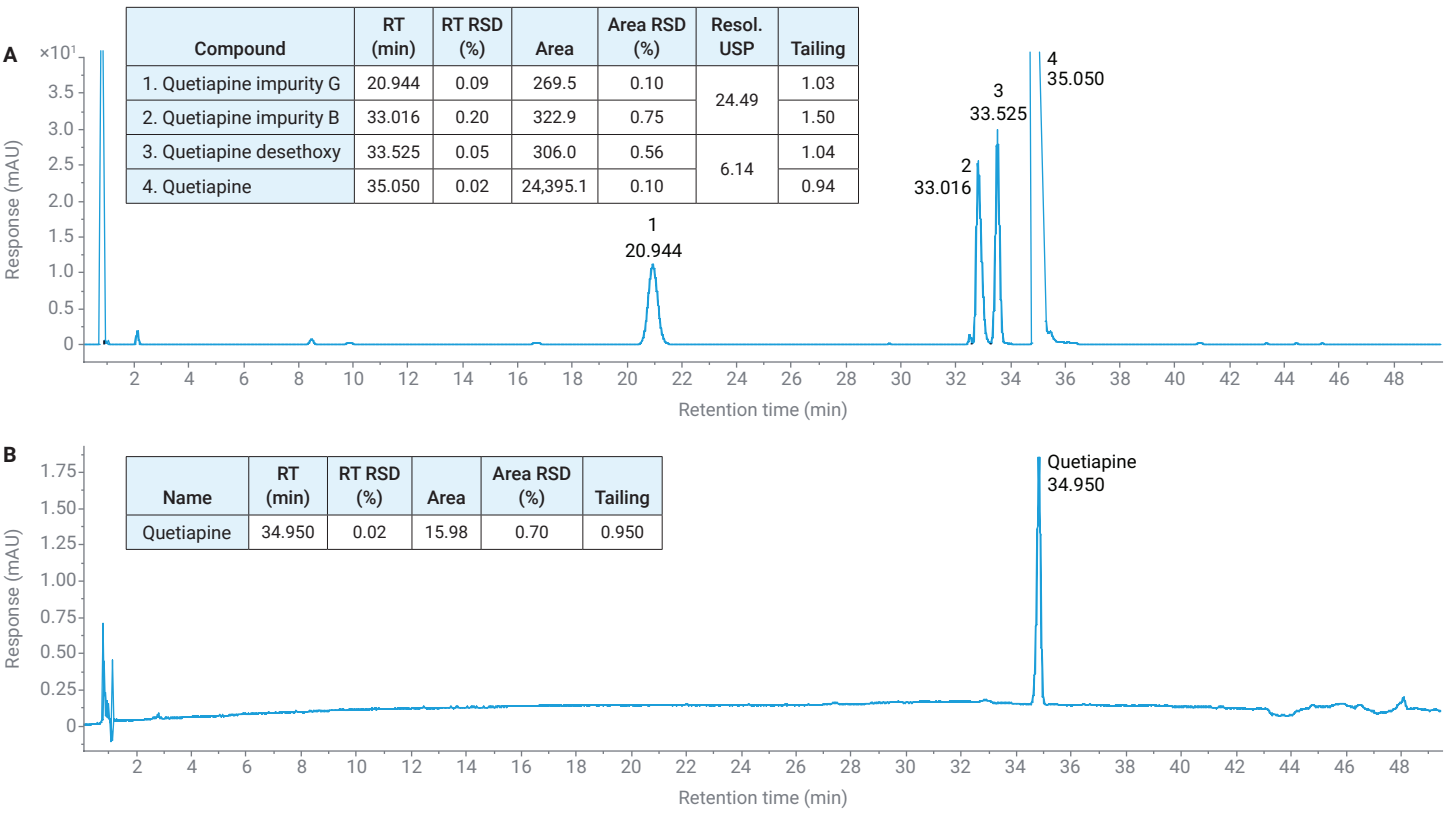
Standard solution: 0.001 mg/mL of USP quetiapine fumarate RS in diluent.

Chemicals and solvents

All solvents were LC grade. Acetonitrile was purchased from Merck (Darmstadt, Germany). Fresh, ultrapure water was obtained from a Milli-Q Integral system equipped with a 0.22 µm membrane point-of-use cartridge (Millipak, EMD Millipore, Billerica, MA, USA). All chemicals (ammonium acetate, ammonia solution 25%) and USP standards (Quetiapine Fumarate RS and Quetiapine System Suitability RS) were purchased from Sigma-Aldrich (Steinheim, Germany).

Results and discussion

In the USP monograph for the determination of quetiapine and its organic impurities, the use of a C8 reversed-phase column, 4.6 × 150 mm, 3.5 µm packing, L7, is mandated. The chromatographic conditions are described in Table 2.[2] Figure 1 shows the results from the analysis of the system suitability solution and the standard solution on a legacy 1100 Series LC system. The requirements of the USP monograph regarding resolution and tailing factors are fulfilled. They require a resolution not lower than (NLT) 4.0 between quetiapine desethoxy and quetiapine, and NLT 3.0 between quetiapine-related compound B and quetiapine-related compound G in system suitability solution. The required tailing factor is not more than (NMT) 2 and the RSD is NMT 5% from quetiapine standard solution.



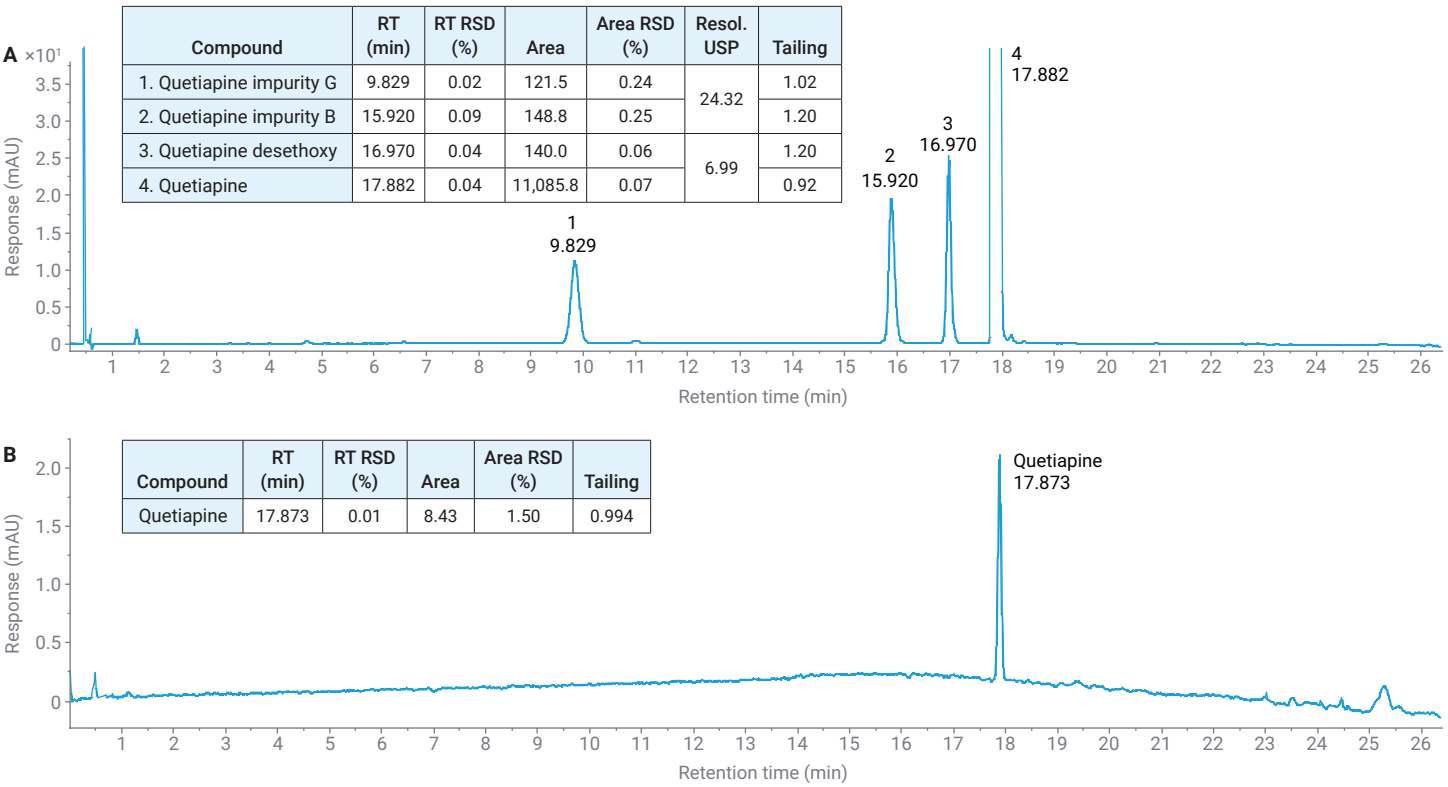
Compound	RT (min)	RT RSD (%)	Area	Area RSD (%)	Resol. USP	Tailing
1. Quetiapine impurity G	20.944	0.09	269.5	0.10	24.49	1.03
2. Quetiapine impurity B	33.016	0.20	322.9	0.75		1.50
3. Quetiapine desethoxy	33.525	0.05	306.0	0.56	6.14	1.04
4. Quetiapine	35.050	0.02	24,395.1	0.10		0.94

Name	RT (min)	RT RSD (%)	Area	Area RSD (%)	Tailing
Quetiapine	34.950	0.02	15.98	0.70	0.950

Figure 1. Analysis of organic impurities of quetiapine as described in the USP monograph. (A) System suitability solution; (B) standard solution.
1. Quetiapine-related compound G; 2. Quetiapine-related compound B; 3. Quetiapine desethoxy; 4. Quetiapine. N = 6 for calculation of RSDs.

To use the 1260 Infinity II Prime LC, the method for the analysis of organic impurities of quetiapine is transferred to UHPLC conditions. The method transfer to an InfinityLab Poroshell EC-C8, 4.6 × 100 mm, 2.7 µm column results in a 14% decrease of the L/dp ratio compared to the original column, which is permitted according to USP chapter <621>. The chromatographic conditions applied for this column are shown in Table 3.

The results from the analysis of the system suitability solution and the standard solution using the InfinityLab Poroshell EC-C8, 4.6 × 100 mm, 2.7 µm column are shown in Figure 2.



Compound	RT (min)	RT RSD (%)	Area	Area RSD (%)	Resol. USP	Tailing
1. Quetiapine impurity G	9.829	0.02	121.5	0.24	24.32	1.02
2. Quetiapine impurity B	15.920	0.09	148.8	0.25		1.20
3. Quetiapine desethoxy	16.970	0.04	140.0	0.06	6.99	1.20
4. Quetiapine	17.882	0.04	11,085.8	0.07		0.92

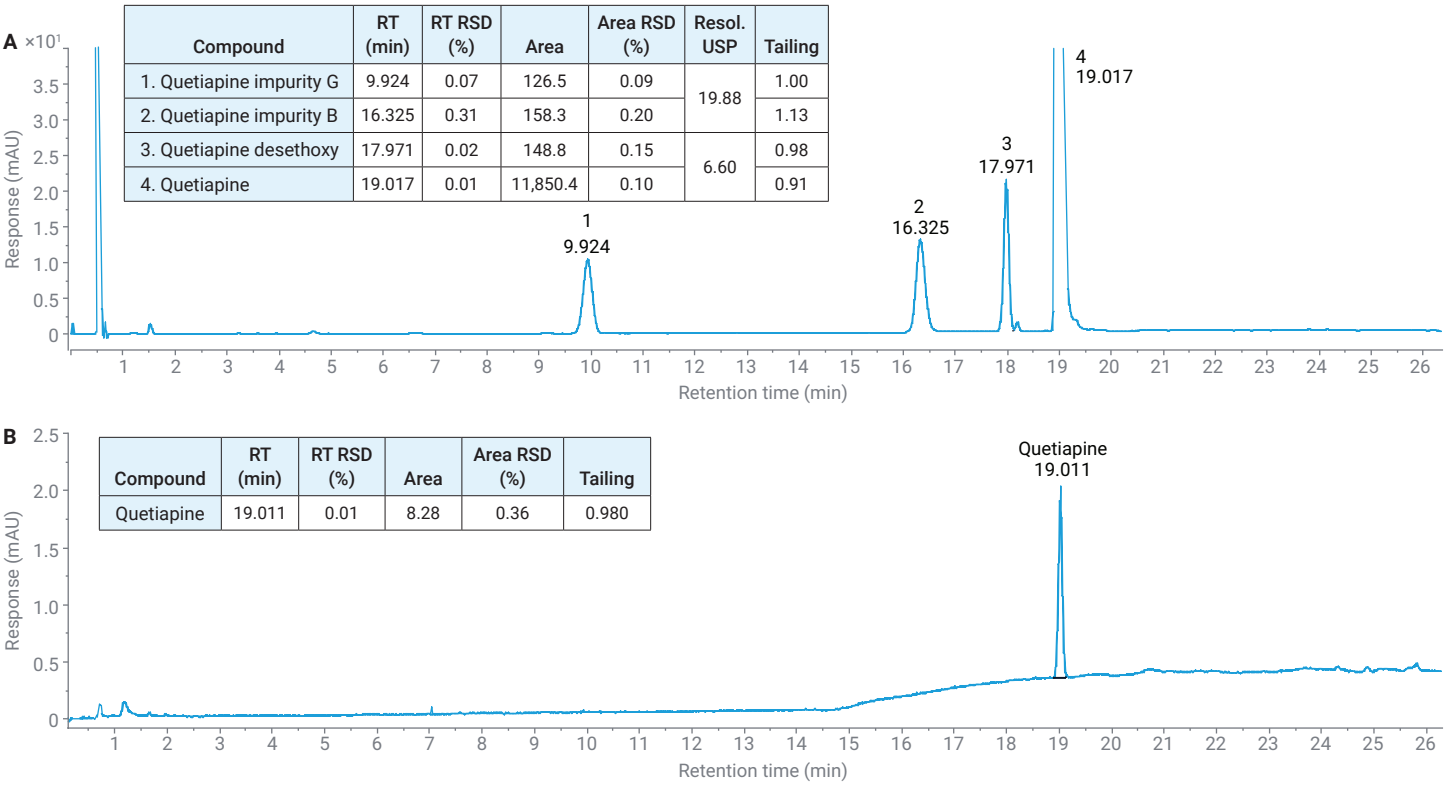
Compound	RT (min)	RT RSD (%)	Area	Area RSD (%)	Tailing
Quetiapine	17.873	0.01	8.43	1.50	0.994

Figure 2. Analysis of organic impurities of quetiapine using the Agilent 1260 Infinity II Prime LC and an Agilent InfinityLab Poroshell EC-C8, 4.6 × 100 mm, 2.7 µm column. (A) System suitability solution; (B) standard solution. 1. Quetiapine-related compound G; 2. Quetiapine-related compound B; 3. Quetiapine desethoxy; 4. Quetiapine. N = 6 for calculation of RSDs.

For further solvent savings, the internal diameter of the column was reduced, remaining within the requirements described in USP chapter <621>. Figure 3 shows the results from analysis of system suitability solution and the standard solution using an InfinityLab Poroshell EC-C8, 2.1 × 100 mm, 2.7 µm column. The adjusted chromatographic conditions are described in Table 4.

The comparison of the analysis of organic impurities of quetiapine as described in the USP monograph and the two UHPLC analyses can be found in Table 5.

The method transfer from a 4.6 × 150 mm, 3.5 µm column to a 4.6 or 2.1 × 100 mm, 2.7 µm column results in a −14% decrease or a +9.3% increase in the L/dp ratio, and is permitted by the requirements described in USP chapter <621>. System suitability and standard solution criteria of the USP monograph on quetiapine are fulfilled by all methods. The transfer to a 4.6 × 100 mm, 2.7 µm column results in 33.5% less solvent consumption per injection, and a 48.5% reduction in analysis time. The transfer to a 2.1 × 100 mm, 2.7 µm column reduces the solvent consumption per injection by 85.9%.



Compound	RT (min)	RT RSD (%)	Area	Area RSD (%)	Resol. USP	Tailing
1. Quetiapine impurity G	9.924	0.07	126.5	0.09	19.88	1.00
2. Quetiapine impurity B	16.325	0.31	158.3	0.20		1.13
3. Quetiapine desethoxy	17.971	0.02	148.8	0.15	6.60	0.98
4. Quetiapine	19.017	0.01	11,850.4	0.10		0.91

Compound	RT (min)	RT RSD (%)	Area	Area RSD (%)	Tailing
Quetiapine	19.011	0.01	8.28	0.36	0.980

Figure 3. Analysis of organic impurities of quetiapine employing the Agilent 1260 Infinity II Prime LC and an Agilent InfinityLab Poroshell EC-C8, 2.1 × 100 mm, 2.7 µm column. (A) System suitability solution; (B) standard solution. 1. Quetiapine-related compound G; 2. Quetiapine-related compound B; 3. Quetiapine desethoxy; 4. Quetiapine. N = 6 for calculation of RSDs.

Table 5. Comparison of the analysis of organic impurities of quetiapine as described in the USP monograph and the UHPLC analyses. Fulfillment of USP requirements is marked in green.

Column and Method			
	Agilent Eclipse XDB-C8, 4.6 × 150 mm, 3.5 µm	**Agilent Poroshell EC-C8, 4.6 × 100 mm, 2.7 µm**	**Agilent Poroshell EC-C8, 2.1 × 100 mm, 2.7 µm**
L/dp	43,000	37,000 (–14%)	47,000 (+9.3%)
Flow Rate	1.5 mL/min	1.97 mL/min	0.41 mL/min
Solvent Consumption per Injection	102 mL	68.95 mL (–33.5%)	14.34 mL (–85.95%)
Run Time	68.00 min	35.00 min (–48.5%)	35.00 min (–48.5%)
System Suitability Requirements in Quetiapine Monograph			
Resolution Between Quetiapine Desethoxy and Quetiapine, NLT 4.0	6.14	6.99	6.60
Resolution Between Quetiapine-Related Compound B and Quetiapine-Related Compound G, NLT 3.0	24.49	24.32	19.88
Standard Solution Requirements in Quetiapine Monograph			
Tailing Factor Quetiapine, NMT 2.0	0.95	0.99	0.98
RT RSD Quetiapine, NMT 5%	0.01	0.01	0.01

Table 6 displays a cost savings calculator, considering the described scenarios for the analysis of organic impurities of quetiapine. You can add the costs, assumptions, and method settings for your own analysis to calculate potential savings when transferring your method to the 1260 Infinity II Prime LC System. For the conventional LC, employing the analysis of organic impurities of quetiapine as described in the USP monograph, the total cost per injection results in $74.40. Replacement with the 1260 Infinity II Prime LC System and transfer of the method to the 2.1 × 100 mm, 2.7 µm column results in a total cost per injection of $63.84.

With the costs and assumptions applied in Table 6, a break-even point of 990 injections, or 10 months, can be calculated, until the higher cost of investment in the 1260 Infinity II Prime LC System (compared to a conventional LC) will be paid off.

Achieving sustainable operations is another important factor that contributes to the profitability of any modern laboratory. With the newly revised USP <621> method, UHPLC—and the more efficient chromatography that it offers—can now even be applied to existing methods in the QC lab. Marked reductions of 85.9% of solvent and close to 50% of analysis times are brought within reach. Replacing legacy equipment can also reduce an analytical lab's instrument footprint and potentially have a positive effect on, for example, energy consumption.[3]

Conclusion

This application note describes the transfer of the USP method for the analysis of quetiapine and its organic impurities to UHPLC conditions according to USP chapter <621>. The newly developed UHPLC method saves up to 85.9% solvent and 48.5% analysis time. For the QC lab, this offers the possibility to maintain analytical certainty in chromatography while realizing the additional goals of lab operations: cost savings and the opportunity to contribute to company-wide sustainability goals.

The replacement of a legacy Agilent 1100 Series LC running under standard USP conditions with an Agilent 1260 Infinity II Prime LC System running under UHPLC conditions will pay off the new instrument after 990 injections. Further, the marked reduction of the environmental footprint through reduced solvent use and analysis time complies with the 12 principles of green chemistry, even under QC conditions.[4]

Table 6. Interactive cost savings calculator for the analysis of organic impurities of quetiapine. Enter your own costs, assumptions, and method settings into the cost savings calculator to see how the 1260 Infinity II Prime LC System can help you save money for your specific analysis.

General Settings	
Solvent Costs per Liter	130 USD
Waste Costs per Liter	5 USD
Labor Costs per Year and Operator	50.000 USD
Linear Depreciation in Years	7
Additional Laboratory Costs per Year	1.000 USD
Daily Operating Hours	8
Weekly Operating Days	5
Yearly Operating Weeks	44
Required Number of Injections per Year (Incl. Blanks, Standards, etc.)	1.200

Instrument Settings	Conventional LC	Agilent 1260 Infinity II Prime LC
Instrument Costs	78.248 USD	88.702 USD
Uptime per Year	90 %	90 %
Maintenance Costs per Instrument and Year	3.875 USD	3.875 USD
Costs per Column	705 USD	732 USD
Column Lifetime: Number of Injections	2.000	2.000
Operators per Instrument	1	1
Consumables Costs per Injection (e.g., Vials, Caps, Filters, Syringes, etc.)	5 USD	5 USD

Method Settings	Conventional LC	Agilent 1260 Infinity II Prime LC
Run Time (Incl. Column Wash and Equilibration)	68,00 min	35,00 min
Flow Rate	1,50 mL/min	0,41 mL/min

Injections and Instruments	Conventional LC	Agilent 1260 Infinity II Prime LC
Maximum Injections per Year	1.397	2.715
Number of Instruments Required	1	1

Financial View	Conventional LC	Agilent 1260 Infinity II Prime LC
Instrument Costs	11.178 USD	12.672 USD
Maintenance Costs	3.875 USD	3.875 USD
Column Costs	705 USD	732 USD
Consumable Costs	6.000 USD	6.000 USD
Solvent Costs	15.912 USD	2.239 USD
Waste Costs	612 USD	86 USD
Additional Laboratory Costs	1.000 USD	1.000 USD
Operator Costs	50.000 USD	50.000 USD
Total Annual Operation	89.282,00 USD	76.604,00 USD
Total Cost per Injection	74,40 USD	63,84 USD

Break-Even Calculations	Agilent 1260 Infinity II Prime LC
Injections	990
Months	10

Sustainability View	Conventional LC	Agilent 1260 Infinity II Prime LC
Solvent Volume Used	122 L	17 L

Reset table

References

1. United States Pharmacopeia General Chapter <621> Chromatography. The National Formulary. Rockville, MD: United States Pharmacopeial Convention, Inc., 1 Dec **2020**.
2. United States Pharmacopeia USP Monograph on Quetiapine. The National Formulary. Rockville, MD: United States Pharmacopeial Convention, Inc., 1 Jan **2023**.
3. Rieck, F. Do You Know the Environmental Impact of Your HPLC? *Agilent Technologies technical overview*, publication number 5994-2335EN, **2022**.
4. Anastas. P. T.; Warner, J. C. Green Chemistry: Theory and Practice; Oxford University Press, **1998**.

www.agilent.com

DE41656484

Printed in the USA, October 15, 2024
5994-6126EN

Agilent
Trusted Answers