Application Note

Biopharma/Pharma

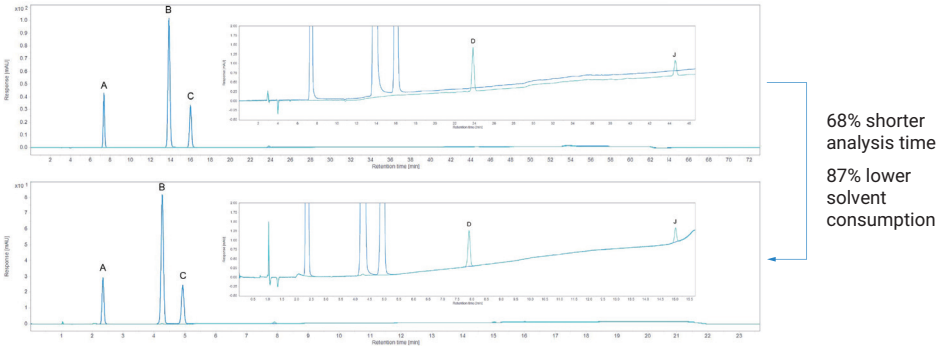
Agilent

Trusted Answers

Reduce the Cost per Injection for Your USP Compendial Method

Method transfer to UHPLC conditions according to USP Chapter 621 requirements using an Agilent 1260 Infinity II Prime LC

Suitable for Agilent
1260 Infinity III LC



Authors

Sonja Schipperges and
Edgar Naegele
Agilent Technologies, Inc.

Abstract

A recent revision of United States Pharmacopeia (USP) chapter 621 on chromatography allows adjustments of chromatographic conditions in gradient elution liquid chromatography (LC). This enables the transfer of gradient methods described in USP monographs to UHPLC conditions. This application note demonstrates method transfer to UHPLC conditions for the analysis of organic impurities of acetaminophen, showing the achieved savings in analysis time and solvent consumption.

This application note also features an interactive cost savings calculator. Use this tool to estimate possible savings made through replacing legacy instrumentation with the Agilent 1260 Infinity II Prime LC and transferring methods to UHPLC conditions.

Notice: 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 general chapters of the USP provide guidelines on activities related to the tests and procedures described in monographs. Among these, chapter 621 provides guidance on chromatography.[1] The chromatographic conditions described in monographs were validated during the elaboration of the monograph. Changes other than the adjustments of chromatographic conditions indicated in USP chapter 621 require revalidation of the procedure. A recent revision of USP chapter 621 includes changes regarding permitted adjustments of chromatographic conditions in gradient elution LC. With this revision, changes in column dimensions, including the transfer from totally porous particle (TPP) to superficially porous particle (SPP) columns, become allowed in gradient elution LC. Table 1 provides an overview of the permitted adjustments of chromatographic conditions for gradient systems.

The permission to adjust chromatographic conditions in gradient elution LC enables the transfer of methods described in USP monographs to UHPLC conditions. This offers the possibility to speed up analysis and reduce solvent consumption, resulting in a lower cost per analysis as well as increased throughput.

This application note demonstrates method transfer to UHPLC conditions for the analysis of organic impurities of acetaminophen (paracetamol) according to the USP monograph.[2] The original gradient separation method is applied using a legacy Agilent 1100 Series LC and is transferred to UHPLC conditions according to the requirements described in USP chapter 621[1] by using the 1260 Infinity II Prime LC in combination with an SPP column. Analysis time, solvent consumption, and resulting cost per injection is compared for the original method and the analysis using UHPLC conditions.

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)

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 length of the column may be modified if the ratio of the column length (L) to the particle size (dp) remains constant or in the range between –25% to +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

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 Plus C8, 4.6 × 250 mm, 5 µm (part number 959990-906)
- Agilent InfinityLab Poroshell 120 EC-C8, 4.6 × 150 mm, 2.7 µm (part number 693975-906)
- Agilent InfinityLab Poroshell 120 EC-C8, 2.1 × 150 mm, 2.7 µm (part number 693775-906)

Chemicals

All solvents were LC grade. Methanol 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). Acetaminophen, acetaminophen-related compound C (N-(2-hydroxyphenyl) acetamide), acetaminophen-related compound D (N-phenylacetamide), and acetaminophen-related compound J (N-(4-chlorophenyl)acetamide) were purchased from Sigma-Aldrich (Steinheim, Germany). Acetaminophen-related compound B (N-(4-hydroxyphenyl) propanamide) was obtained from LGC (Wesel, Germany). Acetic acid was obtained from Fluka (Steinheim, Germany).

Preparation of solutions for system suitability

Solutions for the investigation of system suitability were prepared as described in the USP monograph for acetaminophen (Organic Impurities section).[2] A system suitability solution containing 20 µg/mL of acetaminophen and 80 µg/mL each of acetaminophen-related compounds B and C was prepared in methanol. A standard solution containing 1.25 µg/mL of acetaminophen-related compound D and 0.25 µg/mL of acetaminophen-related compound J was prepared in methanol.

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

Parameter	Value
Column	Agilent ZORBAX Eclipse Plus C8, 4.6 × 250 mm, 5 µm
Solvent	A) Methanol:water:acetic acid (50:950:1, v:v:v) B) Methanol:water:acetic acid (500:500:1, v:v:v)
Gradient	0.00 min – 18% B 8.00 min – 18% B 53.00 min – 100% B 58.00 min – 100% B 59.00 min – 18% B 73.00 min – 18% B Stop time: 73 min
Flow Rate	0.900 mL/min
Temperature	40 °C
Detection	254 nm/4 nm, reference 360 nm/100 nm 10 Hz
Injection	Injection volume: 5.00 µL

Table 3. Method for analysis of organic impurities of acetaminophen: transfer to a 4.6 × 150 mm, 2.7 µm column.

Parameter	Value
Column	Agilent InfinityLab Poroshell 120 EC-C8, 4.6 × 150 mm, 2.7 µm
Solvent	A) Methanol:water:acetic acid (50:950:1, v:v:v) B) Methanol:water:acetic acid (500:500:1, v:v:v)
Gradient	0.00 min – 18% B 2.59 min – 18% B 17.17 min – 100% B 18.79 min – 100% B 19.12 min – 18% B 23.65 min – 18% B Stop time: 23.65 min
Flow Rate	1.670 mL/min
Temperature	40 °C
Detection	254 nm/4 nm, reference 360 nm/100 nm 20 Hz
Injection	Injection volume: 3.00 µL

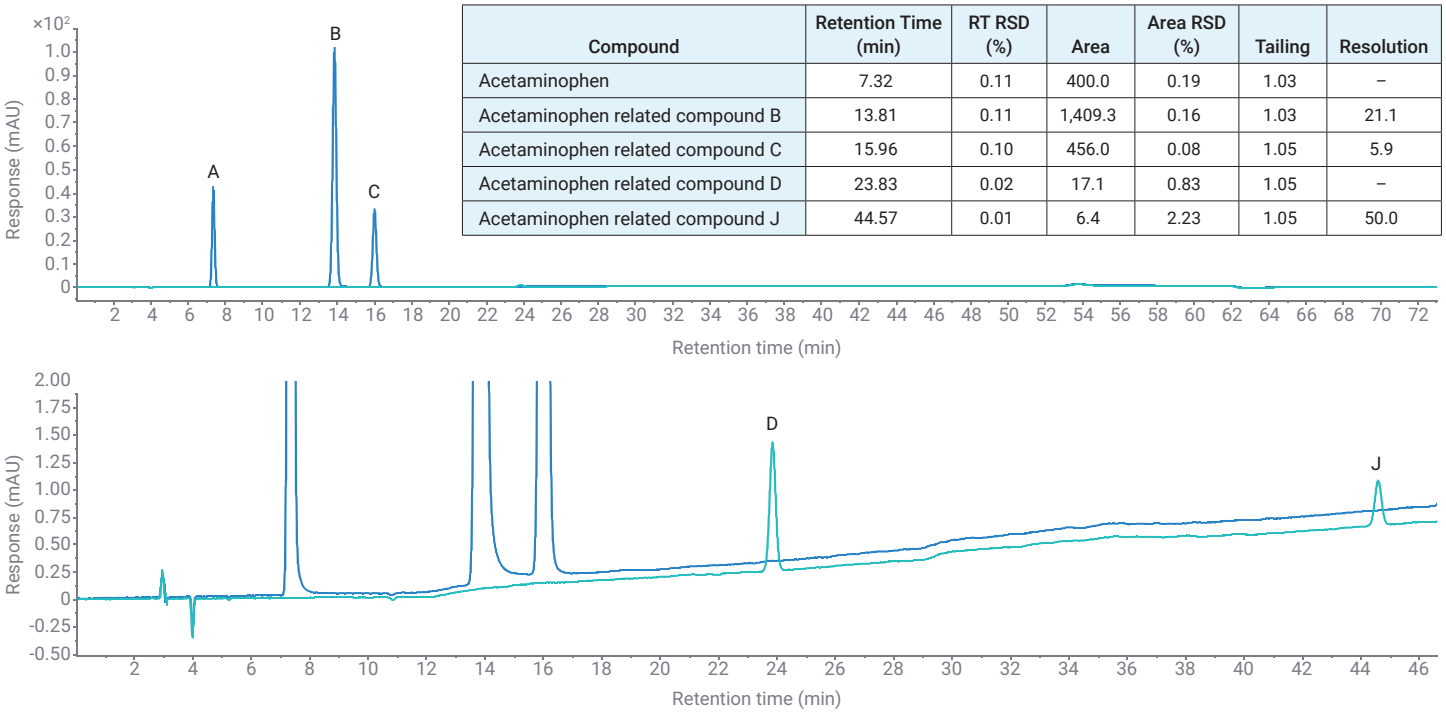
Table 4. Method for analysis of organic impurities of acetaminophen: transfer to a 2.1 × 150 mm, 2.7 µm column.

Parameter	Value
Column	Agilent InfinityLab Poroshell 120 EC-C8, 2.1 × 150 mm, 2.7 µm
Solvent	A) Methanol:water:acetic acid (50:950:1, v:v:v) B) Methanol:water:acetic acid (500:500:1, v:v:v)
Gradient	0.00 min – 18% B 2.59 min – 18% B 17.17 min – 100% B 18.79 min – 100% B 19.12 min – 18% B 23.65 min – 18% B Stop time: 23.65 min
Flow Rate	0.350 mL/min
Temperature	40 °C
Detection	254 nm/4 nm, reference 360 nm/100 nm 20 Hz
Injection	Injection volume: 0.63 µL

Results and discussion

The USP monograph on acetaminophen (paracetamol) prescribes the use of a 4.6 × 250 mm, 5 µm packing L7 column in combination with the chromatographic conditions described in Table 2 for the analysis of organic impurities.[2] Figure 1 shows the results from the analysis of the system suitability and the standard solution using a ZORBAX Eclipse Plus C8, 4.6 × 250 mm, 5 µm column on a legacy 1100 Series LC system.

Using the 1260 Infinity II Prime LC, the analysis of organic impurities of acetaminophen is transferred to UHPLC conditions. The transfer to an InfinityLab Poroshell 120 EC-C8, 4.6 × 150 mm, 2.7 µm column results in an 11% increase in the L/dp ratio compared to the original column and is, therefore, permitted according to USP chapter 621. The adjusted chromatographic conditions applied for this column are shown in Table 3.

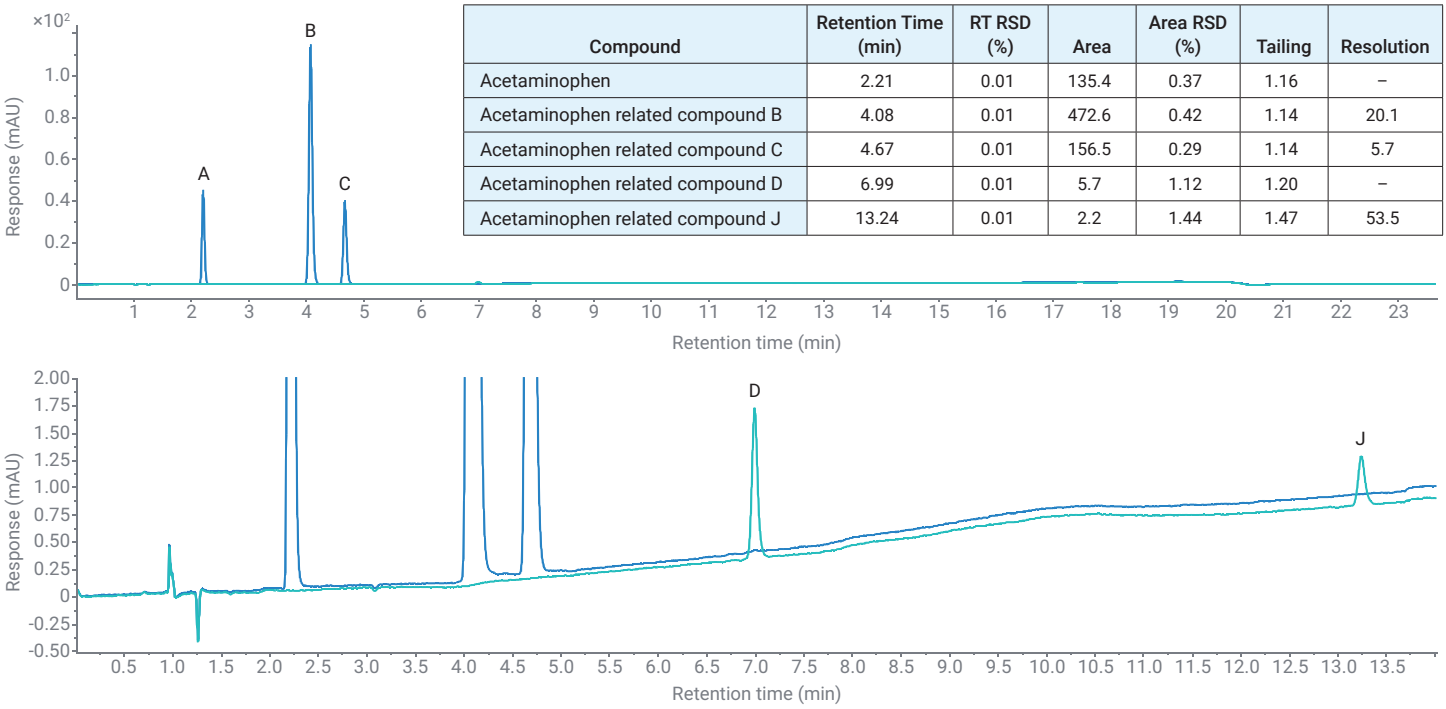


Compound	Retention Time (min)	RT RSD (%)	Area	Area RSD (%)	Tailing	Resolution
Acetaminophen	7.32	0.11	400.0	0.19	1.03	–
Acetaminophen related compound B	13.81	0.11	1,409.3	0.16	1.03	21.1
Acetaminophen related compound C	15.96	0.10	456.0	0.08	1.05	5.9
Acetaminophen related compound D	23.83	0.02	17.1	0.83	1.05	–
Acetaminophen related compound J	44.57	0.01	6.4	2.23	1.05	50.0

Figure 1. Analysis of organic impurities of acetaminophen as described in the USP monograph. Blue: system suitability solution; turquoise: standard solution. (A) acetaminophen; (B) acetaminophen-related compound B; (C) acetaminophen-related compound C; (D) acetaminophen-related compound D; (J) acetaminophen-related compound J. N = 6 for calculation of RSDs.

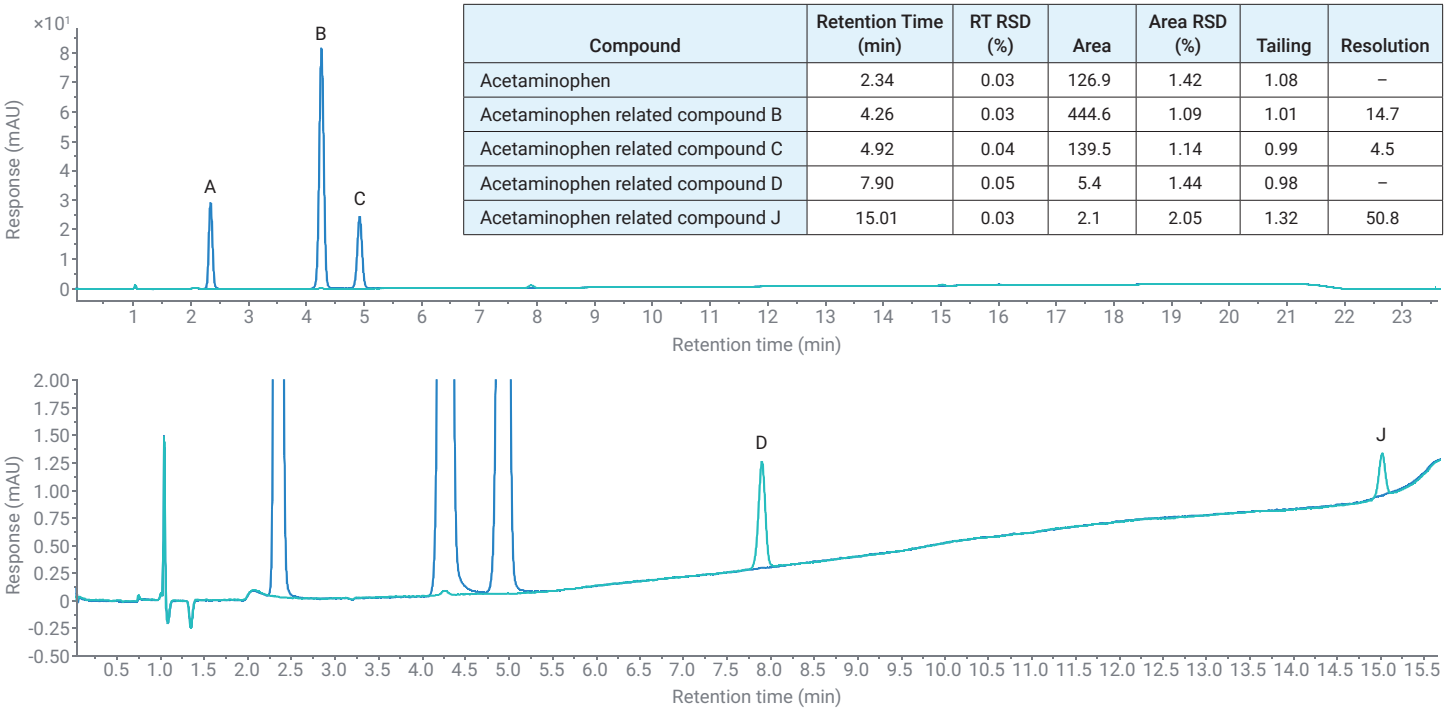
The results from the analysis of the system suitability and the standard solution using the InfinityLab Poroshell 120 EC-C8, 4.6 × 150 mm, 2.7 µm column are shown in Figure 2.

To enable further solvent savings, the internal diameter of the column used can also be adjusted within the requirements described in USP chapter 621. Figure 3 shows the results from the analysis of the system suitability and the standard solution using an InfinityLab Poroshell 120 EC-C8, 2.1 × 150 mm, 2.7 µm column together with the adjusted chromatographic conditions described in Table 4 on the 1260 Infinity II Prime LC.



Compound	Retention Time (min)	RT RSD (%)	Area	Area RSD (%)	Tailing	Resolution
Acetaminophen	2.21	0.01	135.4	0.37	1.16	–
Acetaminophen related compound B	4.08	0.01	472.6	0.42	1.14	20.1
Acetaminophen related compound C	4.67	0.01	156.5	0.29	1.14	5.7
Acetaminophen related compound D	6.99	0.01	5.7	1.12	1.20	–
Acetaminophen related compound J	13.24	0.01	2.2	1.44	1.47	53.5

Figure 2. Analysis of organic impurities of acetaminophen using the Agilent 1260 Infinity II Prime LC and an Agilent InfinityLab Poroshell 120 EC-C8, 4.6 × 150 mm, 2.7 µm column.* Blue: system suitability solution; turquoise: standard solution. (A) acetaminophen; (B) acetaminophen-related compound B; (C) acetaminophen-related compound C; (D) acetaminophen-related compound D; (J) acetaminophen-related compound J. N = 6 for calculation of RSDs.
* The analysis of organic impurities of acetaminophen using the 1260 Infinity II Prime LC and an InfinityLab Poroshell 120 EC-C8, 4.6 × 150 mm, 2.7 µm column with the chromatographic conditions shown in Table 3 results in a maximum pressure close to the maximum pressure applicable for the column. A reduction of the maximum pressure by approximately 40 bar can be obtained by a 5 °C increase of the column temperature, which is permitted within USP 621 requirements. At this increased column temperature, the system suitability criteria described in the USP monograph on acetaminophen are also fulfilled (data not shown).



Compound	Retention Time (min)	RT RSD (%)	Area	Area RSD (%)	Tailing	Resolution
Acetaminophen	2.34	0.03	126.9	1.42	1.08	–
Acetaminophen related compound B	4.26	0.03	444.6	1.09	1.01	14.7
Acetaminophen related compound C	4.92	0.04	139.5	1.14	0.99	4.5
Acetaminophen related compound D	7.90	0.05	5.4	1.44	0.98	–
Acetaminophen related compound J	15.01	0.03	2.1	2.05	1.32	50.8

Figure 3. Analysis of organic impurities of acetaminophen using the Agilent 1260 Infinity II Prime LC and an Agilent InfinityLab Poroshell 120 EC-C8, 2.1 × 150 mm, 2.7 µm column. Blue: system suitability solution; turquoise: standard solution. (A) acetaminophen; (B) acetaminophen-related compound B; (C) acetaminophen-related compound C; (D) acetaminophen-related compound D; (J) acetaminophen-related compound J. N = 6 for calculation of RSDs.

Table 5 presents a comparison between the analysis of organic impurities of acetaminophen described in the USP monograph and the two UHPLC analyses. The transfer from a 4.6 × 250 mm, 5 µm column to a 4.6 or 2.1 × 150 mm, 2.7 µm column results in an 11% increase in the L/dp ratio and is, therefore, possible within the requirements described in USP chapter 621. The system suitability criteria laid out in the USP monograph on acetaminophen are fulfilled by all methods.

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

Column and Method			
	Agilent Eclipse Plus C8, 4.6 × 250 mm, 5 µm	**Agilent InfinityLab Poroshell 120 EC-C8, 4.6 × 150 mm, 2.7 µm**	**Agilent InfinityLab Poroshell 120 EC-C8, 2.1 × 150 mm, 2.7 µm**
L/dp	50,000	55,556 (+11%)	55,556 (+11%)
Flow Rate	0.90 mL/min	1.67 mL/min	0.35 mL/min
Run Time	73.00 min	23.65 min (–67.6%)	23.65 min (–67.6%)
Solvent Consumption per Injection	65.7 mL	39.5 mL (–39.9%)	8.3 mL (–87.4%)
System Suitability Requirements in Acetaminophen Monograph			
Tailing Factor for Acetaminophen-Related Compound D: NMT 2.0	1.05	1.20	0.98
Resolution Between Acetaminophen and Acetaminophen-Related Compound B: NLT 2.0	21.1	20.1	14.7
Resolution Between Acetaminophen-Related Compound B and Acetaminophen-Related Compound C: NLT 1.5	5.9	5.7	4.5
Relative Standard Deviation for Acetaminophen-Related Compound D: NMT 5.0%	0.83	1.12	1.44

The transfer to a 4.6 × 150 mm, 2.7 µm column results in a 67.6% reduction of analysis time and 39.9% less solvent consumption per injection. Using a 2.1 × 150 mm, 2.7 µm column, the solvent consumption per injection can even be reduced by 87.4%.

When replacing a legacy 1100 Series LC, the replacement could either be a conventional LC or a UHPLC such as the 1260 Infinity II Prime LC. Table 6 represents an interactive cost savings calculator considering these two scenarios for the analysis of organic impurities of acetaminophen. Add costs, assumptions, and method settings for your own analysis to calculate potential savings when transferring your method to the 1260 Infinity II Prime LC. For the conventional LC, using the analysis of organic impurities of acetaminophen described in the USP monograph, the total cost per injection results in $69.93 USD. The replacement by the 1260 Infinity II Prime LC and transfer of the method to the 2.1 × 150 mm, 2.7 µm column results in a total cost per injection of $63.76 USD.

With the costs and assumptions laid out in Table 6, a break-even point of 1,978 injections or 20 months can be calculated, until the higher acquisition cost of the 1260 Infinity II Prime LC compared to a conventional LC is paid off.

Table 6. Interactive cost savings calculator for the analysis of organic impurities of acetaminophen. Enter your own costs, assumptions, and method settings to see possible savings when using the Agilent 1260 Infinity II Prime LC for your specific analysis.

General Settings	
Solvent Costs per Liter	6 USD
Waste Costs per Liter	180 USD
Labor Costs per Year and Operator	98.000 USD
Linear Depreciation in Years	87
Additional Laboratory Costs per Year	6.800 USD
Daily Operating Hours	8
Weekly Operating Days	5
Yearly Operating Weeks	44
Required Number of Injections per Year (Incl. Blanks, Standards, etc.)	6.000

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	644 USD	585 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)	25,00 min	8,50 min
Flow Rate	1,00 mL/min	0,21 mL/min

Injections and Instruments	Conventional LC	Agilent 1260 Infinity II Prime LC
Maximum Injections per Year	3.801	11.181
Number of Instruments Required	2	1

Financial View	Conventional LC	Agilent 1260 Infinity II Prime LC
Instrument Costs	1.799 USD	1.020 USD
Maintenance Costs	7.750 USD	3.875 USD
Column Costs	1.932 USD	1.755 USD
Consumable Costs	30.000 USD	30.000 USD
Solvent Costs	900 USD	64 USD
Waste Costs	27.000 USD	1.928 USD
Additional Laboratory Costs	6.800 USD	6.800 USD
Operator Costs	196.000 USD	98.000 USD
Total Annual Operation	272.181,00 USD	143.442,00 USD
Total Cost per Injection	45,36 USD	23,91 USD

Break-Even Calculations	Agilent 1260 Infinity II Prime LC
Injections	488
Months	1

Reset table

Conclusion

Method transfer to UHPLC conditions for the analysis of organic impurities of acetaminophen according to the USP monograph within the requirements described in USP chapter 621 enables a 67.6% reduction of analysis time and 87.4% solvent savings per injection. When replacing a legacy Agilent 1100 Series LC running the analysis of organic impurities of acetaminophen, the replacement by an Agilent 1260 Infinity II Prime LC and method transfer to UHPLC conditions will be paid off after only 1,978 injections compared to the replacement by a conventional LC.

References

1. USP general chapter <621> Chromatography, official as of 1-Dec-2022, https://online.uspnf.com/uspnf/current-document/1_GUID-6C3DF8B8-D12E-4253-A0E7-6855670CDB7B_6_en-US?source=emailLink (accessed 30 January 2023).
2. USP Monograph on Acetaminophen, official as of 1-Jan-2023, https://online.uspnf.com/uspnf/current-document/1_GUID-33AD0880-7404-4169-BDD5-F74D808EE77F_5_en-US?source=emailLink&highlight=Acetaminophen (accessed 30 January 2023).

www.agilent.com

DE51831229

Printed in the USA, October 15, 2024
5994-5897EN

Agilent
Trusted Answers