White Paper

Agilent
Trusted Answers

Understanding the Latest Revisions to USP <621>

Adoption of the revised guidance for analytical method transfers and modernization of LC methods

Authors

Rongjie Fu, Manu Grover, Rob Freeman, and William Long
Agilent Technologies, Inc.

Abstract

Modernization of LC methods is key in lifecycle management of analytical procedures. United States Pharmacopeia (USP) General Chapter <621> allows method adjustments and transfers, making it easier for labs to modernize original USP methods. The revised version of USP <621>, which became effective in December 2022, has been updated to meet industry needs. The USP <621> revisions allow a change in gradient methods, as well as a change from totally porous silica-based analytical columns to superficially porous particle-based columns. These changes were not permitted in previous versions. This white paper outlines such revisions to USP <621> and demonstrates the associated benefits of modernization with respect to increased laboratory throughput and operational cost savings with several case studies.

Introduction

The USP's compendial methods and General Chapter on Chromatography USP <621> are the main guides for analysts to analyze active pharmaceutical ingredients (APIs), finished products, excipients, and so on. These compendial methods intend to achieve system suitability parameters while running across different labs in different time zones using different technologies. Variations in laboratory environment, changes in technology, the need for modernizing old monographs, lifecycle management, and so on, all lead to a demand for modifications in these compendial methods to achieve system suitability results. Therefore, under these scenarios, USP <621> plays a pivotal role.

Currently, there is demand for method transfers from traditional columns and LC methods to modern columns and UHPLC technology. The guidelines in USP General Chapter <621> provide a clearer definition of permissible adjustments during method transfers that meet system suitability requirements. The revised version of USP <621>, which became effective in December 2022, has been updated significantly to meet industry needs. For example, it allows a change in gradient methods, which was not permitted before.

This white paper presents an overview of the revisions to USP <621> and summarizes the changes and criteria for LC method transfer according to the revised USP <621> guidelines. A few case studies for method transfers that depict method modernization are also discussed. Significant improvement in lab productivity and cost savings were achieved by modernizing original USP methods that use classic column technology to newer and smaller particle technology.

USP compendial methods

With over 200 years' history, the USP is a global leader in building trust in medicines, supplements, and foods by setting standards that help ensure quality and safety. The USP is published in a combined volume with the National Formulary (NF) as the USP-NF. The current version, USP-NF 2023, Issue 1, became official on May 1, 2023, covering over 4,900 monographs, of which there are approximately 1,500 monographs for APIs, 450 for excipients, and over 2,500 for finished dosage forms. In addition to monographs, the USP-NF also features more than 330 General Chapters that provide clear, step-by-step guidance for assays, tests, and procedures used in monographs.

USP standards are widely recognized in the U.S. and many other countries worldwide. They are used in more than 140 countries by regulatory agencies and manufacturers to ensure that products are of the appropriate identity, as well as strength, purity, and consistency. The compendial methods of monographs are standardized methods and specification testing for APIs and finished products. The LC method is one of the most popular methods included in each monograph for assaying the starting material and each type of dosage form. These methods are validated test methods that are designed for specific formulated products and APIs, and they are more readily adopted by testing laboratories than those developed from scratch.

USP <621> recent changes, effective December 2022 (latest revision April 2023)

Pharmaceutical companies and several regulatory laboratories often employ USP methods to analyze APIs and finished products. Analysts follow and adopt USP guidance for method development and transfer, including between different instruments or laboratories. Also, analysts implement modernization of the existing USP methods without making significant changes that would require revalidation. By ensuring successful method transferability, pharmaceutical companies can reproduce methods in other labs or with partners such as contract research or manufacturing organizations (CROs and CMOs). Successful method transfer is also a critical step in increasing routine analytical throughput. Following certain method transfer rules, it is possible to transfer traditional HPLC methods to modern UHPLC methods, thereby increasing laboratory throughput and saving operating costs. The method transfer can be achieved following the USP General Chapter <621> guidelines for method adjustments. The <621> guidelines provide a clearer definition of permissible adjustments that meet system suitability requirements. System suitability is an integral part of HPLC methods, as it verifies that the system is adequate for the intended analysis. Each HPLC method in a given monograph may have its own specific system suitability requirements; this is important because if the requirements are not successfully met, the results for that analysis are invalid.

The General Chapter <621> is one of the most important USP General Chapters. It is a quality standard for the pharmaceutical industry, ensuring consistency in chromatography procedures and terms, as well as defining allowable method adjustments. It is also a part of the USP's harmonization initiative, which involves the USP working with other organizations, including the European Pharmacopeia (EP) and Japanese Pharmacopeia (JP), to achieve the international harmonization of pharmacopeial standards.

General Chapter <621> describes general procedures, definitions, and calculations of common parameters and general applicable requirements for system suitability. On December 1, 2022, the newly harmonized General Chapter <621> (Chromatography) of the USP became official. The main changes in the revised USP <621> relate to definitions and allowable adjustments.

Major changes in USP <621>

A summary of the major changes in USP <621> is as follows:

- New definitions are introduced, including for "plate height" and "plate number", as well as definitions regarding size exclusion chromatography, including for "total mobile phase time" and "distribution constant."
- Formulas for "plate number" (previously referred to as "plate count") and "resolution" have been modified to use half height. "Tangent width" no longer appears in the updated USP <621>.
- "Tailing factor" has been renamed as "symmetry factor."
- Signal-to-noise is now calculated using a range of noise that is five times the width at half height of the peak.
- Changes are allowed to gradient chromatographic conditions.

New allowable adjustments in USP <621>

In the previous USP <621>, adjustments to gradients were not allowed. New allowable adjustments to gradients have now been defined in the revised USP <621>. These changes now mean that there is greater alignment between gradient and isocratic tests. The allowable adjustment changes in the updated USP <621> could have a significant impact on the productivity of pharmaceutical labs. Previously, LC methods in the USP may have been adopted easily; they often used older column technology that includes traditional 5 µm particles with long-length columns. Due to the low efficiency per unit length of these types of columns, analysis times are often long. However, with advancements in column technology—specifically the use of smaller particles and superficially porous particles (SPPs) packed in shorter columns—these long analysis times can now be reduced.

Now, both isocratic and gradient methods are allowed to be transferred to the modernized columns according to the USP <621> revisions. This is especially important for pharmaceutical manufacturers who are looking for ways to improve their productivity and save money.

Adjustments to chromatographic conditions

The chromatographic conditions described have been validated during the elaboration of the monograph. The various parameters of a chromatographic test may be adjusted without fundamentally modifying the pharmacopeial analytical procedures.

Changes other than those indicated require revalidation of the procedure.[1] The methods of monographs can be transferred from totally porous particle (TPP) columns to TPP columns or SPP columns. There are four method transfer scenarios according to the new USP <621>. A summary of allowed adjustments for LC methods is shown in Table 1.

Other than the four listed method transfer scenarios, there are still other possible transfers from SPP to TPP columns or SPP to SPP columns. Remember: if SPP columns are involved in any part of the adjustment, follow the rules dictating a transfer to an SPP column for an isocratic or gradient method.

Table 1. Allowable adjustments as per USP General Chapter <621> after December 1, 2022.

Parameters	Method Transfer			
	TPP to TPP column	TPP to SPP Column	TPP to TPP column	TPP to SPP Column
	Isocratic Mode		Gradient Mode	
Stationary Phase	No changes allowed			
Column Dimensions (Particle Size, Length)	L/dp: −25 to 50%	N: −25 to 50%	L/dp: −25 to 50%	$\left(\frac{t_R}{W_h}\right)^2$: −25 to 50%
Column ID	Flexible			
Gradient Time	N/A		Adjust each segment of the gradient $t_{G2} = t_{G1} \times \left(\frac{F_1}{F_2}\right) \times \frac{[L_2 \times dc_2^2]}{[L_1 \times dc_1^2]}$	
Flow Rate	Based on column id and particle size: $F_2 = F_1 \times [(dp_1 \times dc_2^2)/(dp_2 \times dc_1^2)]$ An additional change in flow rate of ±50% is permitted		Based on column id and particle size: $F_2 = F_1 \times [(dp_1 \times dc_2^2)/(dp_2 \times dc_1^2)]$	
Injection Volume	Based on column dimension: $V_2 = V_1 \times [(L_2 \times dc_2^2)/(L_1 \times dc_1^2)]$			
Column Temperature	±10 °C		±5 °C	
Mobile Phase pH	±0.2 units			
Salt Concentration	Within ± 10%, if the permitted pH variation is met			
Ratio of Components in Mobile Phase	Minor component (≤50%): ±30% relative, but cannot exceed ±10% absolute; may only adjust one minor component in ternary mixtures.		The principal peaks elute within ±15% of the retention times obtained with the original conditions; this requirement does not apply when the column dimensions are changed. The composition of the mobile phase and the gradient are such that the first peaks are sufficiently retained, and the last peaks are eluted.	
Wavelength of UV-Vis Detector	No changes allowed			

Agilent LC columns

In USP <621>, there is a complete list of packings (L), phases (G), and supports (S) used in USP-NF tests and assays in USP-NF reagents, indicators, and solutions—chromatographic columns. This list is intended to be a convenient reference for the chromatographer in identifying the pertinent chromatographic column specified in the individual monograph.

Agilent offers both TPP columns (the ZORBAX family[2]) and SPP columns (the InfinityLab Poroshell 120 family[3]) with different USP designations, as shown in Table 2. These column types are all based on the Rx-silica support. Rx-silica particles are ultrapure spherical particles, which is necessary for achieving good peak shape with basic compounds. These particles also have a consistent pore size, accounting for more efficient peaks (higher plates). The silica is fully hydroxylated, offering the highest population of silanols that are favorable to basic compounds, thus overall improving peak shape. Rx-silica is manufactured using a sol-gel process, where silica beads agglutinate in a nonaqueous environment to form the final silica particle. This process is used for maximum particle strength and lifetime.

Table 2. USP designation of Agilent ZORBAX and InfinityLab Poroshell 120 columns.

USP Designation	Agilent ZORBAX Columns	Agilent Poroshell 120 Columns
L1	Eclipse Plus C18 Eclipse XDB-C18 RRHD Eclipse Plus PAH SB-C18 Extend-C18 Rx-C18	EC-C18 Aq-C18 SB-C18 HPH-C18 CS-C18
L3	HILIC Plus Rx-SIL	HILIC
L7	Eclipse Plus C8 Eclipse XDB-C8 SB-C8 Rx-C8	EC-C8 SB-C8 HPH-C8
L10	Eclipse XDB-CN SB-CN	EC-CN
L11	Eclipse Plus Phenyl-Hexyl Eclipse XDB-Phenyl SB-Phenyl	Phenyl-Hexyl
L43		PFP
L45		Chiral-CD
L56	SB-C3	
L60	Bonus-RP	Bonus-RP
L63		Chiral-T
L86		HILIC-OH5
L88		Chiral-V
L96	SB-Aq	SB-Aq
L114		HILIC-Z

The ZORBAX family offers all advantages of TPP columns, such as increased retention, loadability, and resistance to sample solvents. The ZORBAX family is available in different particle sizes including 1.8, 3.5, and 5 µm, and several columns in 7 µm for preparative LC. One of the most valuable assets of the ZORBAX family of HPLC columns is the scalability of methods between particle sizes. This allows a quick and reliable transfer of methods from original USP methods to shorter columns of smaller particle size while keeping the L/dp value within the range of –25 to 50%.

The development of SPPs has led to the possibility of method transfer from larger 5 µm TPP columns to SPP columns. Agilent provides SPP columns of different particle sizes, including 1.9, 2.7, and 4 µm Poroshell 120 columns.

The efficiency of SPP columns is higher than TPP columns of the same particle size due to the short mass-transfer distance and substantially narrower particle-size distribution. The 2.7 µm Poroshell 120 column provides similar efficiency to sub-2 µm TPP columns, while generating half the backpressure. The 1.9 µm Poroshell 120 column provides 20% higher efficiency of sub-2 µm TPP columns, and the 4 µm column provides twice the efficiency of a 5 µm column.

Following the new USP <621> guidance, LC methods can be transferred from 5 µm or other particle-size TPP columns to SPP columns if the N value is within the range of –25 to 50% for isocratic methods, and if the ratio is within –25 to 50% for gradient methods. Keeping a similar selectivity is important when transferring from one method to another as it ensures similar resolution and efficiency after method adjustment, meeting system suitability.

Agilent has traditional ZORBAX chemistries that are also developed on Poroshell 120 particles to offer simplified method transfer from TPP to SPP columns. This is particularly true when columns like the Agilent InfinityLab Poroshell 120 EC-C18 and ZORBAX Eclipse Plus C18 columns are manufactured to have similar bonding chemistries and use similar retention mechanisms. Agilent provides various Poroshell 120 chemistries that mostly can be found in the ZORBAX family portfolio, which makes method transfer between them easier.[4]

The science behind ZORBAX and Poroshell technology

ZORBAX and Poroshell column technologies are unique in the following features:

- Minimal lot-to-lot variation
- Stringent quality tests
- A uniform and known source of silica
- Scalability across different preparative and analytical platforms

ZORBAX

The ZORBAX column family is an HPLC column family. ZORBAX columns are based on traditional, fully porous particles and offer a high loading capacity and resolution. Including Eclipse Plus, Eclipse XDB, StableBond, Bonus-RP and more, the ZORBAX column portfolio offers a selection of stationary phase chemistries that allows you to fine-tune your selectivity to match your application. Scientists can improve resolution by utilizing sub-2 µm particles to maximize system efficiency and confidently scale up to preparative HPLC or perform easy HPLC method transfer.

Poroshell

InfinityLab Poroshell 120 columns are superficially porous columns for reversed-phase LC separations and offer better efficiency and reliability. Twenty chemistries—from various C18 columns to other unique phases—are available in up to three particle diameters: 1.9, 2.7, and 4 µm. This range of chemistries provides a broad choice of selectivity for scalable LC method development, from traditional HPLC to UHPLC and ultralow dispersion UHPLC.

Case studies on method transfers and modernization

According to the revised USP <621>, there are four commonly known method transfer/modernization scenarios across isocratic and gradient methods. Below are some case studies to explain the concepts, processes, and benefits of this modernization.

Scenario A: Adjustment for isocratic elution

According to the method adjustment in USP <621>, the change in conditions for isocratic elution requires four steps:

1. Adjust the column length and particle size according to either L/dp ratio (for TPP to TPP column) or N (for TPP to SPP column). The ratio remains constant or in the range of –25 to 50% of the prescribed.

2. Adjust the flow rate for changes in particle size and column diameter. The flow rate is adjusted for both the change in column diameter and particle size using Equation 1.

Equation 1.

$$F_2 = F_1 \times [(dp_1 \times dc_2^2)/(dp_2 \times dc_1^2)]$$

F_1 = Flow rate indicated in the monograph (mL/min)

F_2 = Adjusted flow rate (mL/min)

dc_1 = Internal diameter of the column indicated in the monograph

dc_2 = Internal diameter of the column used (mm)

dp_1 = Particle size indicated in the monograph (µm)

dp_2 = Particle size of the column used (µm)

3. Adjust the injection volume using Equation 2.

Equation 2.

$$V_2 = V_1 \times [(L_2 \times dc_2^2)/(L_1 \times dc_1^2)]$$

V_1 = Injection volume indicated in the monograph (µL)

V_2 = Adjusted injection volume (µL)

L_1 = Column length indicated in the monograph (cm)

L_2 = New column length (cm)

dc_1 = Column internal diameter indicated in the monograph (mm)

dc_2 = New column internal diameter (mm)

4. Run the adjusted method and check the system suitability results. Other adjustments in analytical procedure conditions, including mobile phase, temperature, pH, and so on, may be required but must be within the permitted ranges described under system suitability (listed in Table 1).

Case study 1: Method transfer from TPP column to TPP column for isocratic elution

The objective of case study 1 is the modernization of the method to shorter dimensions to increase lab productivity. A typical case for transferring the method from TPP to TPP column for isocratic elution is the USP assay method of amlodipine besylate.[5]

The USP method indicates the use of a 3.9 × 150 mm, 5 µm column with L1 column packing.

According to the method adjustment steps:

1. The same L/dp ratio column with 3 mm internal diameter was used for this method.
2. The flow rate was adjusted according to Equation 1.
   - Because the particle size does not change, the column inner diameter was adjusted as long as the linear velocity is kept constant.
   - Therefore, the flow rate was reduced from 1 to 0.6 mL/min on the 3.9 and 3 mm columns respectively.
3. The injection volume was adjusted according to Equation 2. The calculated volume was 30 µL. Because of the excellent sensitivity of the Agilent 1290 Infinity II DAD, a lower injection volume of 20 µL was used in this white paper.
   - Injection volume can be adjusted if it is consistent with accepted precision, linearity, and detection limits according to the USP <621> guidance.

To reduce analysis time and thus increase productivity, the HPLC column was replaced with a sub-2 µm UHPLC column with the same stationary phase chemistry. Since column dimensions were significantly different, several method parameters were changed.

These changes were also carried out following the previously described steps in accordance with USP guidelines to avoid the need for a complete or partial revalidation of the method.

Table 3 shows a summary of the method settings on a 5 and 1.8 µm column. Table 4 summarizes a comparison of the outcome with different methods. Figure 1 shows a comparison of the HPLC and a UHPLC results.

Conclusion of case study 1

The USP method for amlodipine besylate was transferred from HPLC to UHPLC. As all the changes are within USP <621> limits, there was no requirement for method validation. Method verification may be required. System suitability criteria were evaluated and reached. Analysis time and mobile phase consumption were reduced by 87% and 82% respectively compared to the original HPLC method. It is obvious that laboratory productivity and sample throughput were enhanced.

Table 3. Comparison of HPLC and UHPLC methods used in case study 1.

	Original Method in USP	Method Using HPLC	Method Using UHPLC
Column	Packing L1, 3.9 × 150 mm, 5 µm	Agilent ZORBAX Eclipse Plus C18, 3 × 150 mm, 5 µm (p/n 959993-302)	Agilent ZORBAX Eclipse Plus C18 RRHD, 2.1 × 50 mm, 1.8 µm (p/n 959757-902)
L/dp Ratio	30,000 (–25 to 50%)	30,000	27,778 (–7.4%)
Mobile Phase	Methanol/acetonitrile/buffer 35/15/50, isocratic		
Flow Rate	1 mL/min	0.6 mL/min	0.8 mL/min
Temperature	Not indicated	30 °C	30 °C
Injection Volume*	50 µL	20 µL	10 µL
Detection	UV 237 nm	DAD signal 237/4 nm, reference off, 5 Hz	DAD signal 237/4 nm, reference off, 20 Hz
Analysis Time	Approximately three times the retention of amlodipine	23 min	3.1 min

* Can be adjusted if it is consistent with accepted precision, linearity, and detection limits.

Table 4. Comparison of results obtained with both methods.

	USP Requirements	Method Using HPLC	Method Using UHPLC (Modernized Methods)
Resolution	NLT 8.5	13.3	9.7
Tailing Factor at 5% Height	NMT 2.0	1.14	1.31
Area %RSD Amlodipine (n = 6)	NMT 1.0%	0.03%	0.02%
Area %RSD Amlodipine-Related Compound A (n = 6)	NMT 5.0%	0.29%	0.33%
Retention Time	–	7.52 min	1.02 min
Analysis Time	–	23.00 min	3.10 min (–87%)
Mobile Phase Consumption	–	13.8 mL	2.5 mL (–82%)

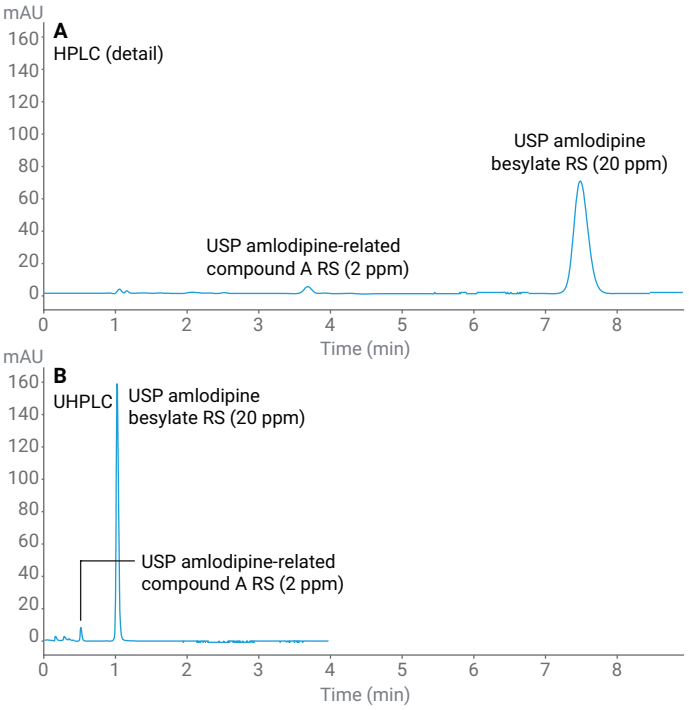

Figure 1. HPLC and UHPLC analysis of system suitability solution for assay method of amlodipine besylate.

Case study 2: Method transfer from TPP column to SPP column for isocratic elution

The objective of case study 2 is the modernization of the method from conventional ZORBAX to new Poroshell columns to improve lab productivity and data quality. A typical case for transferring the method from a TPP to SPP column for isocratic elution is the USP method for diphenhydramine HCl impurities.[6]

Follow the same steps as in case study 1, except: change the column length and particle size using other combinations of L and dp, provided that the plate number (N) is within −25 to 50%. This rule is for the adjustment from TPP to SPP column for isocratic elution.

In this case, the original HPLC method using a 5 µm, 4.6 × 250 mm column with column packing L7 was transferred to a column with new technology: the Agilent InfinityLab Poroshell 120 EC-C8 column (4.6 × 100 mm, 2.7 µm).

Note: Previously, superficially porous columns could be adjusted using the L/dp rule, but under new guidance, only the N rule is acceptable.

Following the L/dp rule, an adjustment from a 5 µm, 250 mm column to a 2.7 µm, 100 mm column would not be an allowed adjustment, as the L/dp is slightly lower than the allowed range. This means a direct comparison of efficiency (N) must be made using the API under investigation in both the original and final adjusted method when using superficially porous columns.

Table 5 displays a summary of the method settings on the 5 and 2.7 µm columns. Table 6 summarizes the comparison of the system suitability results with different methods. Figure 2 shows a comparison of the HPLC and UHPLC chromatograms.

Table 5. Comparison of HPLC and UHPLC methods used in case study 2.

	Original Method in USP	Method Using HPLC	Method Using UHPLC
Column	Packing L7, 4.6 × 250 mm, 5 µm	Agilent ZORBAX Eclipse Plus C8, 4.6 × 250 mm, 5 µm (p/n 959990-906)	Agilent InfinityLab Poroshell 120 EC-C8, 4.6 × 100 mm, 2.7 µm (p/n 695975-906)
Number of Plates of Diphenhydramine	–25 to 50% range of original method	10,334	12,873 (+24.6%)
Mobile Phase	Premix Buffer: 5.4 g/L of monobasic potassium phosphate; adjust with phosphoric acid to a pH of 3.0 Acetonitrile and buffer (35:65)		
Flow Rate	1.2 mL/min	1.2 mL/min	1.5 mL/min
Temperature	Not indicated	25 °C	25 °C
Injection Volume*	10 µL	10 µL	4 µL
Detection	UV 220 nm	DAD signal 220, reference off, 5 Hz	DAD signal 220 nm, reference off, 40 Hz
Run Time	No less than seven times the retention of diphenhydramine	37.1 min	12.3 min

* Can be adjusted if it is consistent with accepted precision, linearity, and detection limits.

Table 6. Comparison of results obtained with both methods.

	USP Requirements	Method Using HPLC	Method Using UHPLC (Modernized Methods)
NLT 2.0 Between Diphenhydramine-Related Compound A and Diphenhydramine	NLT 2.0	2.85	3.02
Retention Time of Diphenhydramine	–	5.30 min	1.76 min
Run Time	–	37.1 min	12.3 min (–66%)
Mobile Phase Consumption	–	13.8 mL	2.5 mL (–82%)

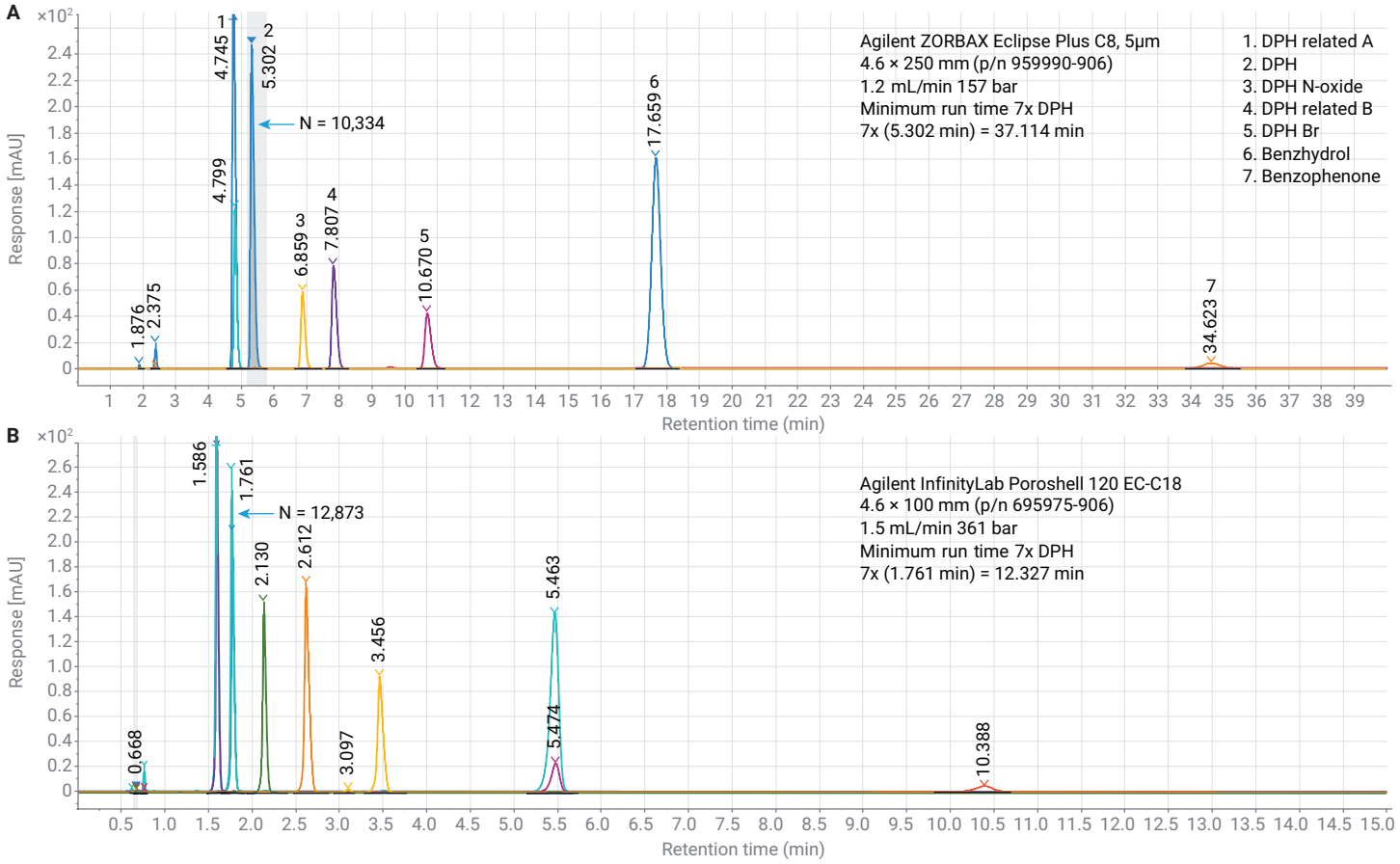

Figure 2. HPLC and UHPLC analysis of system suitability solution for diphenhydramine HCl impurities.

Conclusion of case study 2

In this case, the USP method for diphenhydramine HCl impurities was transferred from a 5 µm TPP column to a 2.7 µm SPP column (no additional method validation required). System suitability criteria were evaluated and reached with both methods. Analysis time and mobile phase consumption were reduced by 66% and 82% respectively compared to the original HPLC method. Increased laboratory productivity and reduction in cost per sample can be achieved using the described approach.

Scenario B: Adjustment for gradient elution

Adjustment for gradient elution was not allowed under USP37-NF32S1 (official Aug 1, 2014), but it is allowed under USP Stage 4 Harmonization (official December 1, 2022).

According to method adjustment in the USP <621>, the change in conditions for gradient elution requires following steps:

1. Adjust the column length and particle size according to the L/dp ratio for TPP to TPP or $(t_R/W_h)^2$ ratio for TPP to SPP. The ratio remains constant or in the range of −25 to 50% of the prescribed ratio.
2. Adjust the flow rate for changes in particle size and column diameter. The flow rate is adjusted for both the change in column diameter and particle size using Equation 1.

Equation 1.

$$F_2 = F_1 \times [(dp_1 \times dc_2^2)/(dp_2 \times dc_1^2)]$$

3. Adjust the injection volume using Equation 2.

   Equation 2.

   $$V_2 = V_1 \times [(L_2 \times dc_2^2)/(L_1 \times dc_1^2)]$$

4. Adjust the gradient time of each segment for changes in column length, diameter, and flow rate using Equation 3.

   Equation 3.

   $$t_{G2} = t_{G1} \times (F_1/F_2) [(L_2 \times dc_2^2)/(L_1 \times dc_1^2)]$$

   t_{G1} = Gradient volume or gradient time (initial)

   t_{G2} = New gradient time

   F = Flow rate

   L × dc = The gradient time for each gradient segment needs to be adjusted to maintain a constant ratio of the gradient volume to the column volume

5. Run the adjusted method and check the system suitability results. Other adjustments in analytical procedure conditions, including mobile phase, temperature, pH, and so on, may be required, but must be within the permitted ranges described under system suitability (see Table 1).

Case study 3: Method transfer from TPP column to TPP column for gradient elution

The objective of case study is the modernization of the method from a conventional ZORBAX column to smaller-particle column to improve lab productivity and data quality.

A typical case for transferring the method from a TPP to TPP column for gradient elution is the related compounds analysis method of Iohexol (USP).[7]

Follow the steps as an adjustment for gradient elution:

1. Change the column length and particle size using other combinations of L and dp, provided that the ratio of the column length (L) to the particle size (dp) remains constant or in the range of −25 to 50% of the prescribed L/dp ratio.
   - This rule is for the adjustment from TPP to TPP column for gradient elution.
   - The USP method indicates the use of a 4.6 x 250 mm, 5 µm column for this analysis, according to the method adjustment steps for gradient methods.
2. Similar L/dp ratio columns (within the range of 37,500 to 75,000), including 3.0 × 150 mm, 3.5 µm and 2.1 × 100 mm, 1.8 µm columns with the same stationary phase chemistry as ZORBAX SB-C18, were used for this method.
3. Adjust the flow rate according to Equation 1. Then, adjust the injection volume according to Equation 2, and adjust the gradient time according to Equation 3.

Table 7 displays a summary of the method settings on the 5, 3.5, and 1.8 µm columns. Table 8 summarizes the comparison of the outcome with different methods. Figure 3 shows a comparison of the HPLC and UHPLC results.

Table 7. Comparison of HPLC and UHPLC methods used in case study 3.

	Original Method in USP	Method Using HPLC	Method Using UHPLC	Method Using UHPLC
Column	Packing L1, 4.6 × 250 mm, 5 µm	Agilent ZORBAX SB-C18, 4.6 × 250 mm, 5 µm (p/n 880975-902)	Agilent ZORBAX SB-C18, 3.0 × 150 mm, 3.5 µm (p/n 863954-302)	Agilent ZORBAX RRHD SB-C18, 2.1 × 100 mm, 1.8 µm (p/n 858700-902)
L/dp Ratio	50,000 (−25 to 50%)	50,000	42,857 (−14.3%)	55,555 (+11.1%)
Mobile Phase	A) Water B) Acetonitrile			
Flow Rate	1 mL/min	1.0 mL/min	0.6 mL/min	0.58 mL/min
Gradient	Time (min) B% 0 1 60 13	Time (min) B% 0 1 60 13 Postrun: 6 min	Time (min) B% 0 1 25 13 Postrun: 4 min	Time (min) B% 0 1 8.6 13 Postrun: 2 min
Temperature	Not indicated	25 °C	25 °C	25 °C
Injection Volume*	10 µL	10 µL	3 µL	1 µL
Detection	UV 254 nm	DAD signal 254/4 nm, reference off, 5 Hz	DAD signal 254/4 nm, reference off, 10 Hz	DAD signal 254 nm/4 nm, reference off, 40 Hz
Analysis Time	60 min	60 min	25 min	8.6 min

* Can be adjusted if it is consistent with accepted precision, linearity, and detection limits.

Table 8. Comparison of results obtained with all methods.

	USP Requirements	Method Using HPLC	Method Using UHPLC (Modernized Methods)	Method Using UHPLC (Modernized Methods)
Resolution Between Related Compounds A and C	NLT 20	51.1	44.0	40.7
Analysis Time	–	60 min	25 min (–58%)	8.6 min (–86%)
Mobile Phase Consumption	–	60.0 mL	15 mL (–75%)	5.0 mL (–92%)

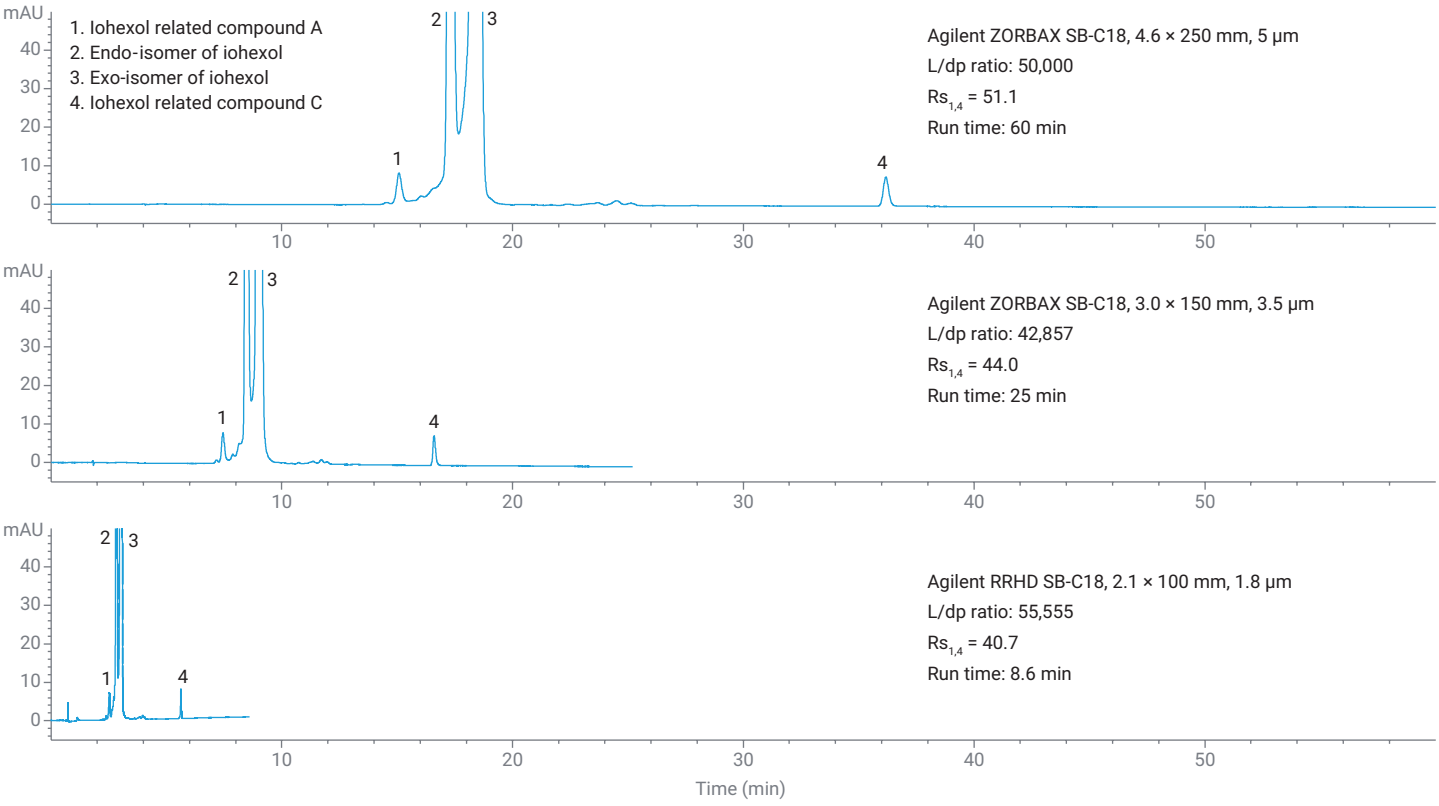

Figure 3. HPLC and UHPLC analysis of system suitability solution for related-compound analysis of iohexol.

Conclusion of case study 3

In this case, the USP method for related compounds analysis of iohexol was transferred from a 5 µm ZORBAX SB-C18 column to a 3.5 or 1.8 µm ZORBAX SB-C18 column without additional method validation. System suitability criteria were evaluated, and all methods showed compliance. Analysis time was reduced by 58% with the 3.5 µm column and 86% with the 1.8 µm column. Mobile phase consumption was also dramatically reduced by 75% with the 3.5 µm column and 92% with the 1.8 µm column. It is obvious that laboratory productivity and sample throughput can be enhanced using the described approach.

Case study 4: Method transfer from TPP column to SPP column for gradient elution

The objective of case study 4 was the modernization of the method from conventional ZORBAX columns to new Poroshell columns to improve lab productivity and data quality. A typical case for transferring the method from TPP column to SPP column for gradient elution is the USP assay method for diphenhydramine HCl.[8]

As an adjustment for gradient elution: change the column length and particle size using other combinations of L and dp, provided that the ratio of $(t_R/W_h)^2$ is within –25 to 50%. This rule is for the adjustment from TPP to SPP column for gradient elution.

In this case, the current assay method for diphenhydramine HCl published in the USP using the ZORBAX Eclipse Plus C8, 4.6 × 250 mm, 5 µm is adjusted within allowable limits to increase sample throughput using the InfinityLab Poroshell 120 EC-C8, 4.6 × 100 mm, 2.7 µm. The gradient was adjusted as the flow rate increased from 1.2 to 2.0 mL/min using Equation 3, and 1.8 mL/min was used.

Table 9 shows a summary of the method settings on the 5 and 2.7 µm columns. Table 10 summarizes the comparison of the system suitability results with different methods. Figure 4 shows a comparison of the HPLC and UHPLC chromatograms.

Table 9. Comparison of HPLC and UHPLC methods used in case study 4.

	Original Method in USP	Method Using HPLC	Method Using UHPLC
Column	Packing L7, 4.6 × 250 mm, 5 µm	Agilent ZORBAX Eclipse Plus C8, 4.6 × 250 mm, 5 µm (p/n 959990-906)	Agilent InfinityLab Poroshell 120 EC-C8, 4.6 × 100 mm, 2.7 µm (p/n 695975-906)
$(t_R/W_h)^2$ of Diphenhydramine	–25 to 50% range of original method	2,410 (1,805 to 3,615)	2,778 (+15.3%)
Mobile Phase	A) Buffer of 5.4 g/L monobasic potassium phosphate; adjust with phosphoric acid to a pH of 3.0 B) Acetonitrile Diluent: Acetonitrile and buffer (35:65)		
Flow Rate	1.2 mL/min	1.2 mL/min	1.8 mL/min
Gradient	Time (min) B% 0 35 4 35 7 80 9 35 13 35	Time (min) B% 0 35 4 35 7 80 9 35 13 35	Time (min) B% 0 35 1.1 35 1.9 80 2.4 35 3.5 35
Temperature	Not indicated	25 °C	25 °C
Injection Volume*	10 µL	10 µL	4 µL
Detection	UV 220 nm	DAD signal 220, reference off, 5 Hz	DAD signal 220 nm, reference off, 40 Hz
Run Time	13 min	13 min	3.5 min

* Can be adjusted if it is consistent with accepted precision, linearity, and detection limits.

Table 10. Comparison of results obtained with both methods.

	USP Requirements	Method Using HPLC	Method Using UHPLC (Modernized Method)
Resolution Between Diphenhydramine-Related Compound A and Diphenhydramine	NLT 1.5	–	2.96
%RSD for Standard Solution	NMT 0.85%	–	0.68%
Tailing Factor	NMT 1.8	–	1.11
Run Time	–	13 min	3.5 min (–73%)
Mobile Phase Consumption	–	15.6 mL	6.3 mL (–60%)

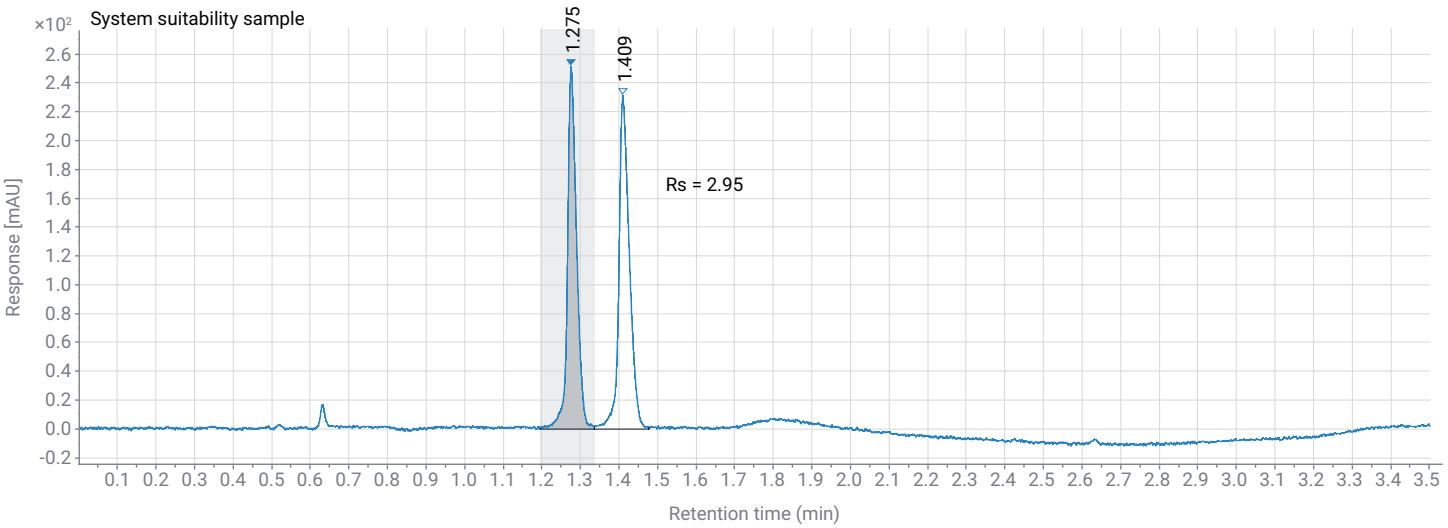

Figure 4. UHPLC analysis of system suitability solution for the diphenhydramine HCl assay.

Conclusion of case study 4

In this case, the USP assay method for diphenhydramine HCl was transferred from a 5 µm TPP column to a 2.7 µm SPP column. System suitability criteria were evaluated, and both methods were in compliance. Analysis time and mobile phase consumption were reduced by 73% and 60% respectively compared to the original HPLC method. It is obvious that laboratory productivity and sample throughput can be enhanced using the described approach.

USP <621> emphasizes: "Caution is necessary when the adjustment results in smaller peak volumes due to a smaller particle size or smaller internal column diameter, a situation that may require adjustments to minimize extra column band broadening by factors such as instrument connections, detector cell volume and sampling rate, and injection volume." Agilent recommends a risk assessment to access cumulative effect of multiple adjustments during modernization and method transfers across column platforms. System suitability is the primary verification for compliance of satisfactory performance.

Considering dwell and dispersion volumes as other major criteria for method transfers

Monographs preferably include an isocratic step before the start of the gradient program. This is so that an adaptation can be made to the gradient time points to account for differences in dwell volume between the system used for analytical procedure development and the actual system used. The revised USP <621> shows how to calculate dwell volume.

Conclusion

USP compendial methods are widely used for pharmaceutical product testing worldwide. Some HPLC methods use older column technologies, which lead to poor reproducibility, poor peak shapes, and inconsistent results. Typically, run times are also long. Newer LC column technologies with regular silica, minimal lot-to-lot variation, including smaller particle size columns and SPP columns, can provide similar or even better results while saving analysis time and mobile phase consumption. The revised USP General Chapter <621> guidance makes the method adjustment or transfer more flexible from traditional 5 µm TPP columns to smaller particle size columns or SPP columns, without additional method validation. The benefits of these method transfers or modernization include time and solvent consumption savings and the related solvent generation savings. These savings will ultimately result in lower lab operational costs and increased laboratory productivity.

References

1. USP Harmonized Standards Home Page. Supplement USP Stage 4 Harmonization, Official, December 1, **2022** (latest revision in April 2023).
2. Agilent ZORBAX family, *Agilent Technologies poster*, publication number 5994-2212EN.
3. Agilent InfinityLab Poroshell 120 family, *Agilent Technologies poster*, publication number 5991-9013EN.
4. Transfer of Methods between Poroshell 120 EC-C18 and ZORBAX Eclipse Plus C18 Columns, *Agilent Technologies technical overview*, publication number 5990-6588EN, **2011**.
5. Vanhoenacker, G.; Steenbeke, M.; Sandra, K. HPLC to UHPLC Transfer of USP Method for Amlodipine Besylate Using the Agilent 1290 Infinity II LC, *Agilent Technologies application note*, publication number 5991-6540EN, **2016**.
6. Long, W. J. A Simple Conversion of the USP Method for Diphenhydramine HCl Impurities to the Agilent InfinityLab Poroshell 120 EC-C8 Column, *Agilent Technologies application note*, publication number 5994-5391EN, **2022**.
7. Fu, R. Gradient Method Transfer of the Iohexol USP Monograph HPLC Method for Related Compounds to Smaller Particle Size ZORBAX Columns, *Agilent Technologies application note*, publication number 5994-6544EN, **2023**.
8. Long, W. J. A Simple Conversion of the USP Assay Method for Diphenhydramine HCl to the Agilent InfinityLab Poroshell 120 Column EC-C8, *Agilent Technologies application note*, publication number 5994-5400EN, **2022**.

www.agilent.com

DE48655502

Printed in the USA, August 30, 2023
5994-6618EN

Agilent
Trusted Answers