Application Note

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Gradient Method Transfer of the Iohexol USP Monograph HPLC Method for Related Compounds to Smaller Particle Size ZORBAX Columns

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Abstract

The original USP monograph HPLC method of related-compounds analysis for iohexol was transferred to smaller particle size 3.5 and 1.8 µm Agilent ZORBAX columns following the newly revised U.S. Pharmacopeia (USP) General Chapter <621> guidelines. The original method uses a gradient separation with a 4.6 × 250 mm, 5 µm column and requires 60 minutes for the analysis. The analysis time was reduced from 60 to 25 minutes when the method was transferred to the Agilent ZORBAX SB-C18, 3.0 × 150 mm, 3.5 µm column (58% reduction in analysis time and 75% reduction in solvent consumption). Furthermore, analysis time was reduced from 60 to 8.6 minutes when the method was transferred to the Agilent ZORBAX RRHD SB-C18 column, 2.1 × 100 mm, 1.8 µm (86% reduction in analysis time and 92% reduction in solvent consumption), without method revalidation. All system suitability requirements were met while achieving significant reductions in both analysis time and solvent consumption.

Introduction

In most USP monographs, there are HPLC methods for testing raw materials and formulated products. These methods have been the routine analysis techniques for generic pharmaceutical manufacturers. These methods mostly employ older column technology that includes conventional 5 µm particle columns. Due to the low efficiency of these columns, longer columns (e.g., 150 or 250 mm long) are often required, leading to long analysis times. An analyst's main job is to reproduce methods in the USP and, in many cases, transfer methods between different instruments or laboratories. Analysts also need to modernize existing USP methods without making any significant changes that would require revalidation. The previous USP <621> only allowed method transfer from conventional 5 µm columns to smaller particle size columns for isocratic methods. The current USP <621> guidelines, revised in December 2022, now allow for the modernization of gradient methods both using totally porous particle (TPP) columns with smaller particle sizes, and superficially porous particle (SPP) columns.[1]

In this application note, the original related-compounds testing method in the USP that uses 4.6 × 250 mm, 5 µm columns for iohexol[2] was transferred to smaller particle size columns under the current USP <621> guidelines. The original method was first run on a 5 µm ZORBAX SB-C18 column, and then transferred to a 3.5 or 1.8 µm ZORBAX SB-C18 column.

Experimental

Instruments and materials

An Agilent 1260 Infinity II LC system was used for 5 and 3.5 µm columns, with 0.17 mm tubing throughout, and an Agilent 1290 Infinity II LC was used for the 1.8 µm column, with 0.12 mm tubing throughout. Table 1 shows the instrument configurations.

All reagents and solvents were HPLC grade. Acetonitrile, iohexol, and related compounds were purchased from Anpel Laboratory Technologies (Shanghai, China). Water was purified using an ELGA PURELAB Chorus system (High Wycombe, UK). The system suitability solution was prepared according to the USP monograph of iohexol.

The following columns were used:

- Agilent ZORBAX SB-C18, 4.6 x 250 mm, 5 µm (p/n 880975-902)
- Agilent ZORBAX SB-C18, 3.0 x 150 mm, 3.5 µm (p/n 883975-302)
- Agilent ZORBAX SB-C18, 2.1 x 100 mm, 1.8 µm (p/n 858700-902)

Table 1. Instrument configurations.

Agilent 1260 Infinity II LC System	
Agilent 1260 Infinity II binary pump (G7112B)	Agilent 4-position/10-port valve, 600 bar (p/n 5067-4279
Agilent 1260 Infinity II multisampler (G7167A)	Agilent vial, screw top, amber with write-on spot, certified, 2 mL (p/n 5182-0716) Agilent cap, screw, blue, PTFE/red silicone septa (p/n 5182-0717)
Agilent 1260 Infinity II multicolumn thermostat (G7116A)	Agilent InfinityLab Quick Connect heat exchanger, standard (G7116-60015) Heater and column: Agilent InfinityLab Quick Connect fitting assembly, 105 mm, 0.17 mm (p/n 5067-6166)
Agilent 1260 Infinity II diode array detector WR (G7115A)	Agilent flow cell, 10 mm, 13 µL (p/n G1315-60022)
Agilent OpenLab CDS, version C.01.07	Agilent flow cell, 10 mm, 13 µL (p/n G1315-60022) Agilent long-life deuterium lamp (p/n 2140-0820)
Agilent 1290 Infinity II LC System	
Agilent 1290 Infinity II high speed pump (G7120A)	Agilent InfinityLab Quick Change valve head, 4-position/10-port valve, 1,300 bar (p/n 5067-4233)
Agilent 1290 Infinity II multisampler (G7167B)	Agilent vial, screw top, amber with write-on spot, certified, 2 mL (p/n 5182-0716) Agilent cap, screw, blue, PTFE/red silicone septa (p/n 5182-0717)
Agilent 1290 Infinity II multicolumn thermostat (G7116B)	Agilent InfinityLab Quick Connect heat exchanger, standard (G7116-60015) Heater and column: Agilent InfinityLab Quick Connect fitting assembly, 105 mm, 0.12 mm (p/n 5067-5957)
Agilent 1290 Infinity II diode array detector (G7117B)	Agilent InfinityLab Max-Light cartridge cell (p/n G4212-60008) Agilent long-life deuterium lamp, 40 Hz (p/n 5190-0917)
Agilent OpenLab CDS, version C.01.10	

Results and discussion

Previously, under allowable adjustment guidelines, no adjustment of gradient conditions was allowed without revalidation. Under this rule, any change of column dimension and even slight changes in particle size from the USP method (such as 2.7 versus 2.6 µm) were a cause for revalidation.[3] However, the newly revised USP <621> guidelines, published in December 2022, allow adjustments from TPP columns to TPP columns for gradient methods, provided that the identity of the substituent is not changed, and the other physicochemical characteristics of the stationary phase (i.e., chromatographic support, surface modification, and extent of chemical modification) are similar. The particle size and/or length of the column may be modified, provided that the ratio of L/dp remains constant or in the range of −25 to 50% of the prescribed L/dp ratio. These changes are acceptable without revalidation, provided that system suitability criteria are fulfilled, and selectivity and elution order of the specified impurities to be controlled are demonstrated to be equivalent.

In this application note, the same chemistry of the ZORBAX SB-C18 was used for related compounds analysis in iohexol. The allowable range of L/dp ratio is 37,500 to 75,000. The ratios of the smaller ZORBAX SB-C18, 3.0 × 150 mm, 3.5 µm (42,875) and ZORBAX RRHD SB-C18, 2.1 × 100 mm, 1.8 µm (55,555) columns are both within the range.

In this application note, the particle size was changed. Therefore, the flow rate required adjustment because smaller-particle columns require higher linear velocities for the same performance (as measured by reduced plate height). Flow rate is adjusted for both the change in column diameter and particle size using the following equation:

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

- 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 (mm)
- 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)

A change in column dimensions, and thus in column volume, impacts the gradient volume, which controls selectivity. Gradients are adjusted to the column volume by changing the gradient volume in proportion to the column volume. The new gradient time (t_{G2}) can be calculated from the original gradient time (t_{G1}), the flow rate, and the column dimensions as follows:

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

- t_{G1} = Gradient volume or gradient time (initial)
- t_{G2} = New gradient time
- F = Flow rate
- $L \times dc^2$ = The gradient time for each gradient's segment needs to be adjusted to maintain a constant ratio of the gradient volume to the column volume

As the column dimensions were changed, the following equation may be used for adjusting the injection volume:

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

- 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)

The original method conditions are listed in Table 2. The original method was run on an ZORBAX SB-C18, 4.6 × 250 mm, 5 µm column then transferred to an ZORBAX SB-C18, 3 × 150 mm, 3.5 µm column. Both methods were run on the 1260 Infinity II system with a binary pump. Although the ZORBAX SB-C18, 3 × 150 mm, 3.5 µm column has a smaller particle size and smaller internal column diameter, the 1260 Infinity II LC system with a binary pump was still fit for this method. If the method was transferred to the ZORBAX RRHD SB-C18, 2.1 × 100 mm, 1.8 µm column, peak volume would be significantly reduced; therefore, the extra column and delay volume needed to be minimized. An Agilent 1290 Infinity II LC system was therefore used for the method with the ZORBAX RRHD SB-C18, 2.1 × 100 mm, 1.8 µm column.

Analysis time was reduced by 58% with the 3.5 µm column and 86% with the 1.8 µm column, and 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. The gradient conditions used with the 5 µm column and with 3.5 and 1.8 µm columns are shown in Table 3. The system suitability for all the columns met the requirements, as shown in Table 4.

Table 2. Original LC method conditions for related-compounds analysis in iohexol.

Parameter	Value for Related-Compounds Analysis Method
Column	L1: 4.6 × 250 mm
Mobile Phase	A: Water B: Acetonitrile
Gradient	Time (min) B% 0 1 60 13
Flow Rate	1.0 mL/min
Column Temperature	Not indicated
Injection Volume	10 µL
Detector	254 nm

Table 3. Gradient conditions used for three columns of different particle sizes.

Column	Flow Rate (mL/min)	Gradient	Injection Volume (µL)	Multicolumn Thermostat (°C)	Diode Array Detector
Agilent ZORBAX SB-C18, 4.6 × 250 mm, 5 µm (p/n 880975-902)	1.0	Time (min) %B 0 1 60 13	10	25	254 nm, 5 Hz
Agilent ZORBAX SB-C18, 3.0 × 150 mm, 3.5 µm (p/n 863954-302)	0.6	Time (min) %B 0 1 25 13	3	25	254 nm, 10 Hz
Agilent ZORBAX RRHD SB-C18, 2.1 × 100 mm, 1.8 µm (p/n 858700-902)	0.58	Time (min) %B 0 1 8.6 13	1	25	254 nm, 40 Hz

Table 4. System suitability summary.

Parameter	Retention Time	Resolution	Peak Area
USP System Suitability Requirements	The retention time for the O-alkylated compounds is between 1.1 and 1.4 relative to 1.0 for the exo-isomer of iohexol	The resolution, R, between iohexol related compound A and iohexol related compound C is not less than 20.0	The peak area of iohexol related compound C is 0.5 ±0.1% by comparison to the total area of all the peaks in the chromatogram
Agilent ZORBAX SB-C18, 4.6 × 250 mm, 5 µm	20.25 to 25.78 min	51.1	0.55%
Agilent ZORBAX SB-C18, 3.0 × 150 mm, 3.5 µm	9.91 to 12.61 min	44.0	0.55%
Agilent ZORBAX RRHD SB-C18, 2.1 × 100 mm, 1.8 µm	3.34 to 4.25 min	40.7	0.56%

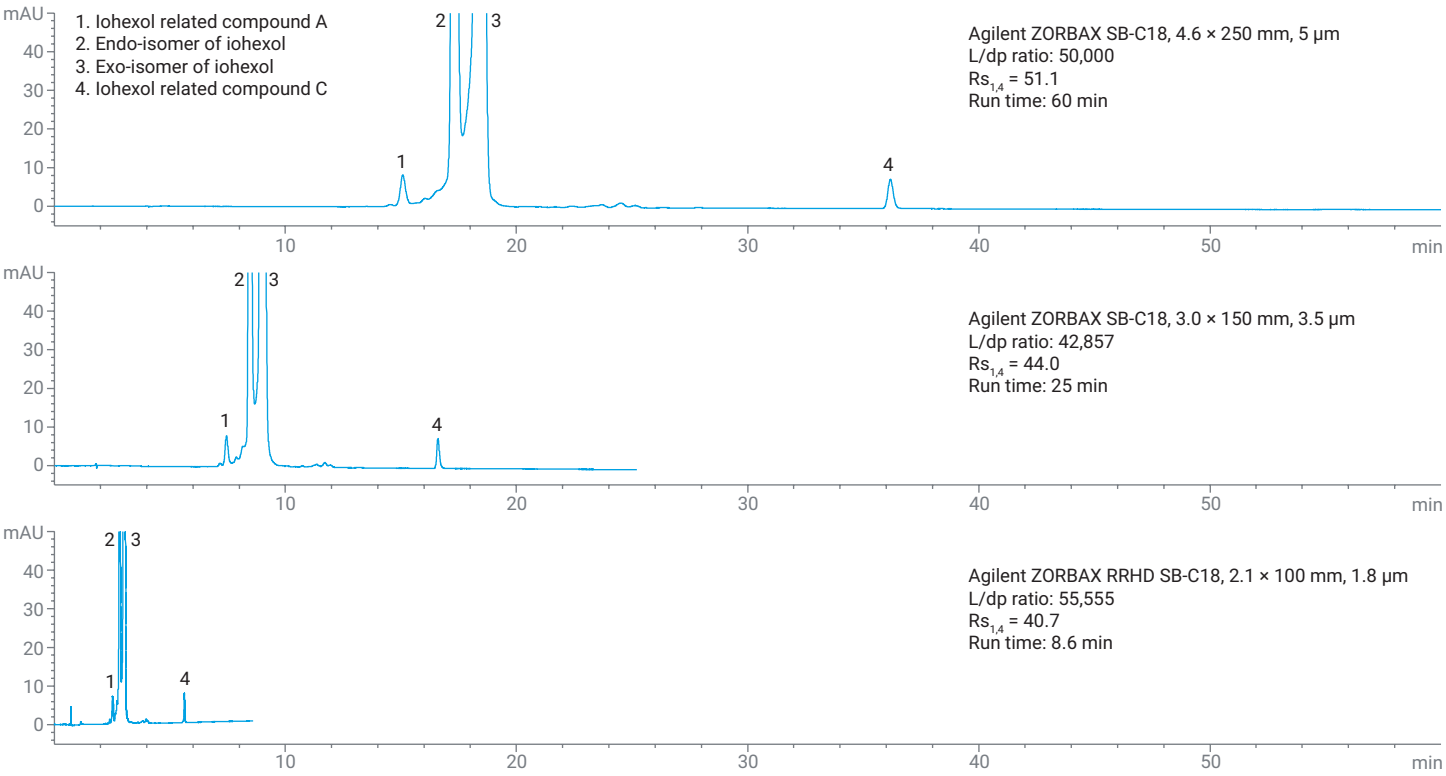

Figure 1. Chromatograms of system suitability solution for related-compounds analysis in iohexol using three columns of different particle sizes.

Conclusion

The USP method of related-compounds analysis for iohexol using conventional 5 µm TPP columns was successfully transferred to smaller particle size columns with the L/dp ratio in the range of –25 to 50%. The methods that used shorter columns with smaller particle sizes provided similar or improved results while significantly reducing analysis times and mobile phase use. These method adjustments are allowable according to the newly revised USP <621> guidelines without needing method revalidation.

References

1. USP Harmonized Standards Home Page. Supplement USP Stage 4 Harmonization, Official, December 1, **2022**.
2. USP 35 Monographs. Iohexol, United States Pharmacopeia: 3534-3536.
3. 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**.

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