Application Note

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A Gradient USP Method Modernization Using Agilent InfinityLab Poroshell 120 Aq-C18

The iohexol USP monograph HPLC method for related compounds was transfered to a superficially porous particle 2.7 µm following the newly revised USP <621> guidelines

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Abstract

The original USP monograph HPLC methods of related compounds analysis for iohexol was modernized to a superficially porous particle (SPP) 2.7 µm column following the newly revised USP <621> guidelines. The original method uses a gradient separation using an Agilent ZORBAX SB-C18, 4.6 × 250 mm, 5 µm column, and requires 60 minutes for analysis. When the method was transferred to an Agilent InfinityLab Poroshell 120 Aq-C18, 4.6 × 100 mm, 2.7 µm column, the analysis time was reduced from 60 to 13 minutes with a 78% reduction in analysis time and 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 technique for generic pharmaceutical manufacturers. These methods mostly employ older column technology that include conventional 5 µm particle columns. Due to the low efficiency of these types of 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. Also, analysts modernized the existing USP methods without making any significant changes, eliminating the need for revalidation. USP only allows method transfer from conventional 5 µm columns to smaller particle size columns for isocratic methods, according to the previous USP <621> guidelines. The current USP <621> guidelines, revised in December 2022, now allow for the modernization of gradient methods using both totally porous particle (TPP, with smaller particle sizes) and superficially porous particle (SPP) columns.[1]

In this application note, the original related compounds testing method using 4.6 × 250 mm, 5 µm columns for iohexol in the USP[2] were transferred to smaller particle size SPP columns under the current USP <621> guidelines. The original method was first run on a 5 µm Agilent ZORBAX SB-C18 column[3], and then transferred to the newly developed InfinityLab Poroshell 120 Aq-C18, 2.7 µm column.

Experimental

Instruments and materials

An Agilent 1260 Infinity II LC system was used with 0.17 mm tubing throughout for both 4.6 × 250 mm and 4.6 × 100 mm columns.

All reagents and solvents were HPLC grade. Acetonitrile, the 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 a USP monograph of iohexol.

Columns

- Agilent ZORBAX SB-C18, 4.6 × 250 mm, 5 µm (part number 880975-902)
- Agilent InfinityLab Poroshell 120 Aq-C18, 4.6 × 100 mm, 2.7 µm (part number 695975-742)

Table 1. Instrument configurations.

Agilent 1260 Infinity II LC system	
Agilent 1260 Infinity II Binary Pump (G7112B)	4-pos/10-port valve 600 bar (p/n 5067-4287)
Agilent 1260 Infinity II Multisampler (G7167A)	Vial, screw top, amber with write-on spot, certified, 2 mL, 100/pk (p/n 5182-0716) Cap, screw, blue, PTFE/red silicone septa, 100/pk (p/n 5182-0717)
Agilent Infinity II Multicolumn Thermostat (MCT)	Standard flow heater (G7116-60015) Heater and column: InfinityLab Quick Connect assembly, 105 mm, 0.17 mm (p/n 5067-6166)
Agilent 1260 Diode Array Detector WR (G7115A)	Flow cell: 10 mm 13 µL flow cell (p/n G1315-60022) Long-life deuterium lamp (p/n 2140-0820)
Agilent OpenLab CDS, Version C.01.07 Software	

Results and discussion

Previously, under adjustment guidelines, no adjustment of gradient conditions was allowed without revalidation. Under this rule, any change of column dimension and even slight particle size from the USP method (such as 2.7 versus 2.6 µm) were cause for revalidation.[4] The newly revised USP <621> guidelines in December 2022 state: "A change from TPP columns to SPP columns is allowed provided 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, must be similar. The particle size and/or length of the column may be modified provided that the ratio of $(t_R/ W_h)^2$ remains constant or in the range between −25 to +50% of the prescribed $(t_R/ W_h)^2$ ratio. These changes are acceptable without revalidation, provided system suitability criteria are fulfilled, and selectivity and elution order of the specified impurities to be controlled are demonstrated to be equivalent." The ratio of $(t_R/ W_h)^2$ and the allowable range are calculated for iohexol-related compound A and iohexol-related compound C that are used to determine the system suitability parameters using the Agilent ZORBAX SB-C18, 4.6 × 250 mm, 5 µm column with the original USP method. The values are shown in Table 2.

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

Parameter	Original in USP	Using UHPLC
Column	Agilent ZORBAX SB-C18, 4.6 × 250 mm, 5 µm (p/n 880975-902)	Agilent InfinityLab Poroshell 120 Aq-C18, 4.6 × 100 mm, 2.7 µm (p/n 695975-742)
$(t_R/W_h)^2$ ratio	3,890 (–25% to 50%) 20,217 (–25% to 50%)	3,023 (–22.3%) 25,290 (25.1%)
Mobile Phase	A: water B: acetonitrile	A: water B: acetonitrile
Gradient	Time (min) B% 0 1 60 13	Time (min) B% 0 1 13 13
Flow rate	1 mL/min	1.85 mL/min
Temperature	25 °C	25 °C
Injection Volume	10 µL	4 µL
Detection	DAD signal 254 nm, ref off 2.5 Hz	DAD signal 254 nm, ref off 40 Hz

In this study, a new InfinityLab Poroshell 120 Aq-C18 column was used for the modernized method. It has been developed based on 2.7 µm SPP with low C18 bonding density and a larger pore size of 120 Å, which enables column use with a high aqueous mobile phase with less retention loss. This is still a USP L1 column, so the InfinityLab Poroshell 120 Aq-C18 columns were allowed to be used for the modernized method. The dimension of 4.6 × 100 mm with a 2.7 µm column was used, because it has similar efficiency with sub-2 µm by 100 mm length, which has comparable efficiency to 250 mm, 5 µm columns. System suitability solution was run with both InfinityLab columns and the ratio of $(t_R/ W_h)^2$ was also calculated for iohexol-related compound A and iohexol-related compound C. The ratios of both columns are all within the allowable range. The values are summarized in Table 2.

In this application note, the particle size was changed, and the flow rate required adjustment, because smaller-particle columns will require higher linear velocities for the same performance (as measured by reduced plate height). Flow rate was 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 (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(s), and the column dimensions as shown in Equation 2.

Equation 2.

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

- 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

Equation 3 can be used for adjusting the injection volume if the column dimensions are changed.

Equation 3.

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

- 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 and new method conditions are shown in Table 2. The methods using 4.6 mm internal diameter columns were run on a 1260 Infinity II LC system with a binary pump. The 4.6 mm internal diameter Poroshell 120 columns fit well with the 1260 Infinity II LC system which has the same maximum pressure of 600 bar. The system suitability for both the columns all met the requirements, which is shown in Table 3. With the modernized method using the InfinityLab Poroshell 120 Aq-C18, 4.6 × 100 mm, 2.7 µm column, analysis time was reduced from 60 to 13 minutes (a time saving of 78%), and solvent consumption was also dramatically reduced by 78%. It is obvious that laboratory productivity and sample throughput can be enhanced using the described approach. Chromatograms are shown in Figure 1.

Table 3. System suitability summary.

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 InfinityLab Poroshell 120 Aq-C18, 4.6 × 100 mm, 2.7 µm (p/n 695975-742)	5.03 to 6.40 min	45.9	0.55%

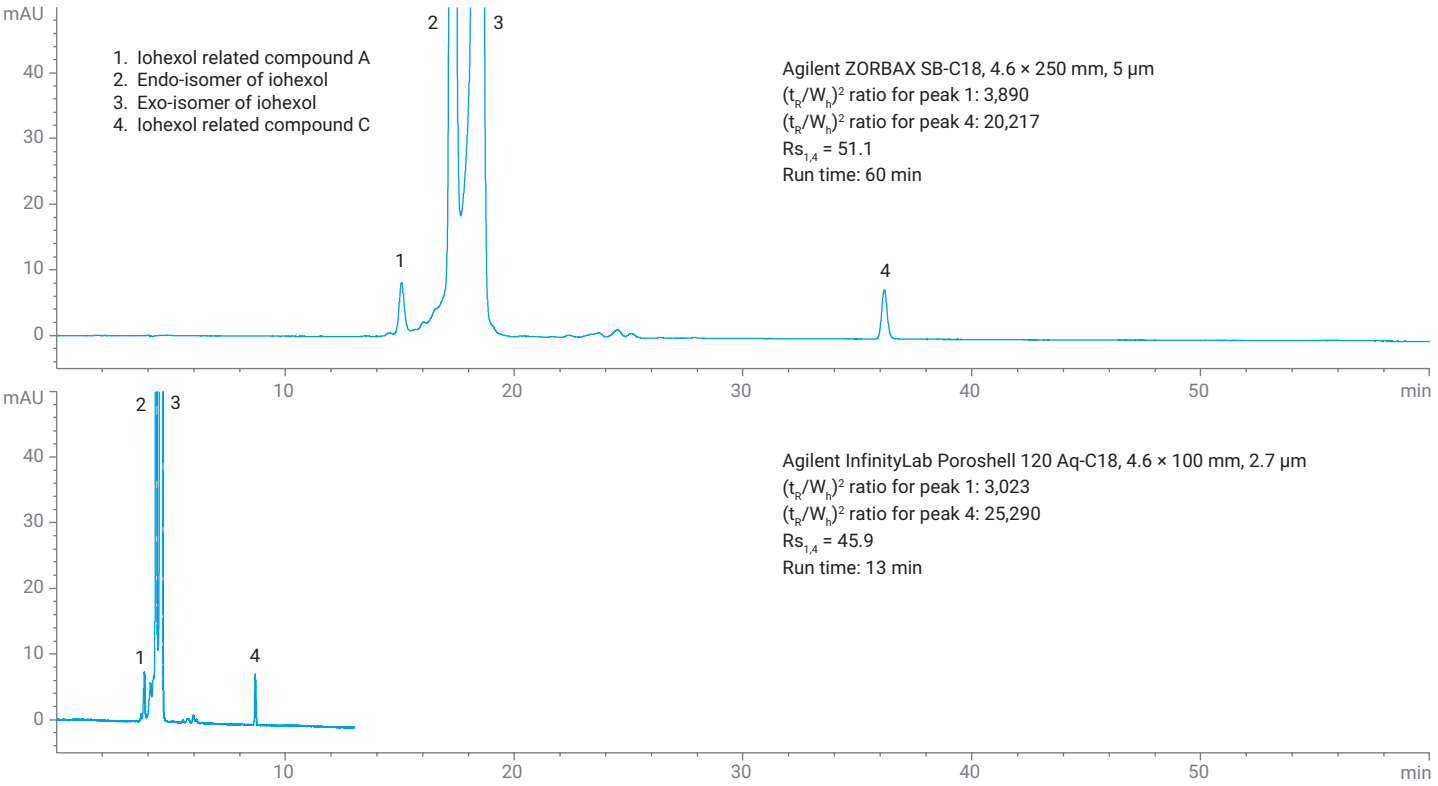

Figure 1. Chromatograms of system suitability solution for related compounds analysis in iohexol using both different particle size columns.

Conclusion

USP methods of related compounds analysis for iohexol using conventional totally porous 5 µm columns have been successfully modernized using the Agilent InfinityLab Poroshell 120 Aq-C18 column. The modernized methods provide similar results while significantly reducing analysis times and solvent usage. Laboratory productivity and sample throughput can be enhanced using this method. The method adjustments are allowable according to the new revised USP <621> guidelines without additional method revalidation.

References

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