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High Performance Liquid Chromatography

Ultra-High Speed Analysis of USP Methods Conforming to Permissible Limits in New USP General Chapter 621

In the General Chapter <621> Chromatography of the U.S. Pharmacopeia (USP), the ranges within which changes to HPLC and GC parameters are permissible are indicated, and as long as the values are within that range, and as long as the system suitability requirements are satisfied, the method can be changed without revalidation. An example of a faster method by which these changes remained within the permissible range was previously introduced in Application News No. L448. The General Chapter 621 was again revised, and the revision has been in effect as of August 1, 2014.
Previously, a reduction of up to 50 % in the column particle size was permitted, but according to the new revisions to the General Chapter 621 that has been in effect, the column particle size can be freely selected. Therefore, even though the current particle size of 5 µm is specified, columns with particle size in the sub-2 µm region may be used, permitting even faster analyses.
Here, we introduce an example of a faster analysis of a USP method in compliance with the new General Chapter 621 using the Nexera X2 ultra high performance liquid chromatograph and the Shim-pack XR-ODS Ⅲ high-speed analytical column.

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Permissible Limits of Modification for HPLC Parameters

Tables 1 and 2 list the permissible limits of the HPLC column-related parameters according to the General Chapter 621 of the previous and new version of the specifications, respectively. These changes apply to the column length, particle size, and flowrate. It should be noted that these changes apply only to isocratic analysis, and are not applicable to gradient analysis.

Table 1 Permissible Limits of HPLC Parameters in the Previous General Chapter 621 (effective until July 31, 2014)

Column diameter	Can be adjusted if the linear velocity is kept constant
Column length	Can be adjusted by as much as ±70 %
Particle size	Can be reduced by as much as 50 %, but cannot be increased.
Flowrate	Can be adjusted based on column cross-sectional area ratio. In addition, can be adjusted by ±50 %.

Table 2 Permissible Limits of HPLC Parameters in the New General Chapter 621 (effective as of August 1, 2014)

Column diameter	Can be adjusted if the linear velocity is kept constant
Column length and particle size	May be modified provided that the ratio of the column length (*L*) to the particle size (*dp*) remains constant or within the range of -25 % to +50 % of the prescribed *L*/*dp* ratio.
Flowrate	Can be adjusted using column cross-sectional area ratio and particle size inverse ratio.* When a change is made from ≧3 µm particle size to <3 µm particle size, or from <3 µm particle size to ≧3 µm particle size, the linear velocity may be changed within a range in which the column efficiency does not decrease more than 20 %.* In addition, it can be adjusted by ±50 %.

* See text

This change is based on the theory that if *L*/*dp* is kept constant, equivalent separation performance will be maintained. For example, if a column of length 150 mm and particle size of 5 µm (*L*/*dp* = 150,000 µm / 5 µm = 30,000) is changed to one of column length 50 mm and particle size 1.6 µm (*L*/*dp* = 31,250), *L*/*dp* can be maintained at +4.2 %, thereby obtaining equivalent separation performance. In addition, the following formula can be used with respect to flowrate.

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

F_1 and F_2 express the original USP monograph flowrate and the modified flowrate, respectively. Also, *dc* and *dp* express the column internal diameter and column packing particle size, respectively. This expression includes two principles. The first is that the flowrate is adjusted in proportion to the column cross-sectional area, or in other words, the linear velocity is kept constant. The second is that because the particle size and optimal flowrate are inversely proportional, the point is to change the flowrate in inverse proportion to the particle size. For example, the USP monograph stipulates that for a 4.6 × 150 mm, 5 µm column, a flowrate of 1.0 mL/min is specified, and if the column is changed to a 2.0 × 50 mm, 1.6 µm column, a flowrate of 0.59 mL/min is required. Further, if the particle size is changed from ≧3 µm to <3 µm, it is permissible to increase the flowrate further as long as the column efficiency does not decrease more than 20 %. Conversely, if a <3 µm particle size is changed to ≧3 µm, the flowrate may need to be decreased so that the column efficiency does not decrease more than 20 %.

Speed Enhancement of a USP Method

Here, we introduce a faster method of the impurity analysis of sulfacetamide as defined in the USP. Sulfacetamide is a type of sulfonamide antibacterial drug. The column specified in the USP for this analysis is the 4.6 × 150 mm, 5 µm, L1 (ODS), using a flowrate of 0.8 mL/min. In this case, if the 2.0 × 50 mm, 1.6 µm, L1, Shim-pack XR-ODS Ⅲ column is used, almost the same *L*/*dp* value is obtained. The flowrate can be calculated as 0.47 mL/min from the formula just introduced. Although analysis of a system suitability test solution, a standard solution, and the sample solution are all required in an actual analysis, here we show the chromatograms obtained from analysis of system suitability test solutions (0.2 mg/mL USP sulfacetamide reference standard, 0.05 mg/mL sulfanilamide USP reference standard). For the HPLC system, the Prominence was used, and for the UHPLC system, the Nexera X2 was used. Table 3 shows the analytical conditions, Fig. 1 shows the chromatograms obtained, and Table 4 shows the system suitability test results. Using the Nexera X2 system and the Shim-pack XR-ODS Ⅲ column, in addition to meeting the system suitability requirements, we were able to shorten the analysis time significantly. In this investigation, not only was the analysis shortened to about 1/10 the time on a per-minute basis, solvent consumption was also reduced to about 1/15 that normally required.

Table 3 Analytical Conditions

System	: (1) Prominence (2) Nexera X2 (Loop injection with 20 µL loop)
Column	: (1) Shim-pack VP-ODS (150 mm L. × 4.6 mm I.D., 4.6 µm) (2) Shim-pack XR-ODS Ⅲ (50 mm L. × 2.0 mm I.D., 1.6 µm)
Mobile Phase	: Methanol / water / acetic acid = 10/89/1 (v/v/v)
Column Temp.	: Ambient
Flowrate	: (1) 0.8 mL/min (2) 0.47 mL/min
Injection Vol.	: (1) 10 µL (2) 2 µL
Detection	: (1) SPD-20AV at 254 nm (2) SPD-M30A at 254 nm
Flow Cell	: (1) Conventional cell (for SPD-20A(V)) (2) Standard cell (for SPD-M30A)

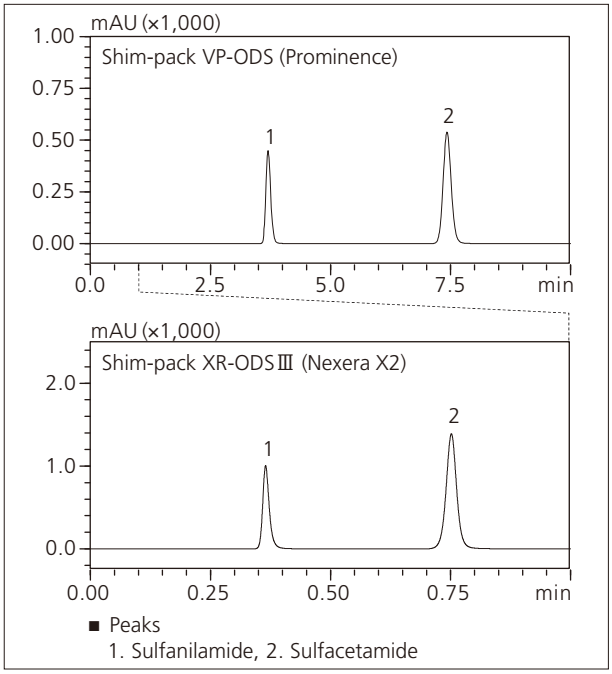

Fig. 1 Chromatograms of System Suitability Test Solution (Upper: VP-ODS with Prominence, Lower: XR-ODS Ⅲ with Nexera X2)

Table 4 Results of System Suitability Test

System suitability requirements		VP-ODS (Prominence)	XR-ODS (Nexera X2)
USP resolution between sulfacetamide and sulfanilamide	≧ 5.0	14.54	12.23
USP tailing factor for sulfacetamide	≦ 1.5	1.09	1.04
Relative standard deviation for sulfacetamide	≦ 2.0 %	Rt 0.015 %	Rt 0.037 %
		Area 0.067 %	Area 0.103 %

A USP Method Performed on Nexera X2

Next, this example shows how analysis of a timolol maleate ophthalmic solution is conducted using the Nexera X2 without changing the analytical conditions specified in the USP. Timolol maleate is a type of non-selective β-blocker. Table 5 shows the analytical conditions used, Fig. 2 shows the chromatogram of the standard solution (0.136 mg/mL timolol maleate) obtained, and Table 6 shows the system suitability test results. Even using the Nexera X2 UHPLC system, it is clear that analysis equivalent to that using the Prominence HPLC system is easily achieved without any problem.

Table 5 Analytical Conditions

System	: (1) Prominence (2) Nexera X2 (Loop injection with 20 µL loop)
Column	: Shim-pack VP-ODS (150 mm L. × 4.6 mm I.D., 4.6 µm)
Mobile Phase	: Sodium phosphate buffer <pH 2.8> / methanol = 65/35 (v/v)
Column Temp.	: 40 °C
Flowrate	: 1.2 mL/min
Injection Vol.	: 10 µL
Detection	: (1) SPD-20AV at 295 nm (2) SPD-M30A at 295 nm
Flow Cell	: (1) Conventional cell (for SPD-20A(V)) (2) Standard cell (for SPD-M30A)

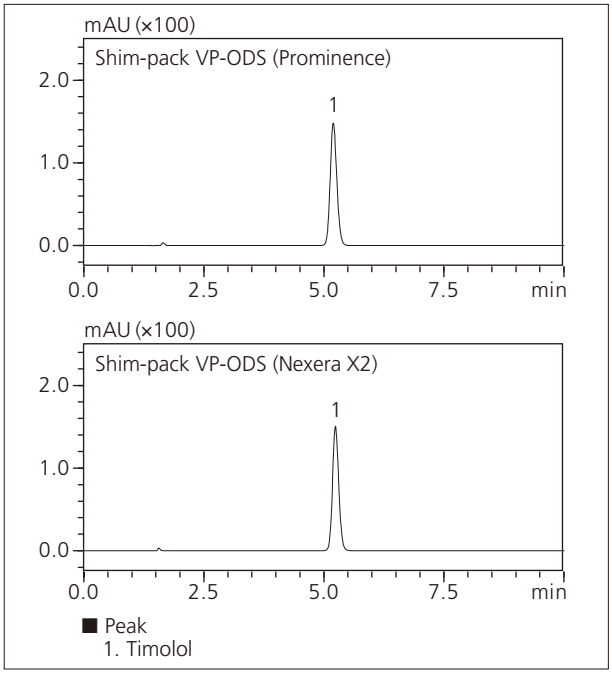

Fig. 2 Chromatograms of Standard Solution (Upper: VP-ODS on Prominence, Lower: VP-ODS on Nexera X2)

Table 6 Results of System Suitability Test

System suitability requirements		VP-ODS (Prominence)	VP-ODS (Nexera X2)
USP tailing factor	≦ 2.0	1.12	1.11
USP column efficiency	≧ 3600	6354	6965
Relative standard deviation	≦ 2.0 %	Rt 0.027 %	Rt 0.082 %
		Area 0.034 %	Area 0.062 %

Conclusion

This study demonstrated that, in accordance with the new USP General Chapter 621, even higher-speed analysis is possible with the Shim-pack XR-ODS Ⅲ and Nexera X2. Also, because the Nexera X2 can be used not only for UHPLC analysis, but for HPLC analysis as well, it is also suitable for those who are running traditional USP methods on a standard HPLC system, and are considering the adoption of a UHPLC system for higher-speed analysis of USP methods in the future..

[References]
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