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Ultra-high Speed Analysis of USP methods conforming to the New USP General Chapter 621 Allowed Limits

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Introduction

The United States Pharmacopeia (USP) defines allowed adjustments of HPLC and GC parameters in the general chapter 621 <chromatography>. If the system suitability is met, method parameters can be changed within the allowed limits without revalidation. The general chapter 621 was revised in the first supplement to USP37-NF32 published on February 1st 2014 and became official on August 1st 2014. The feature of the new general chapter 621 is that a column packed with small particles can be used if column length and particle ratio (*L/dp*) is kept constant between the designated and modified column. This enables high speed analysis of USP methods more than ever.

Key parameters of allowed HPLC adjustments in the current and new general chapter 621 were shown in Table 1 and 2, respectively. The allowed adjustments for column length and particle size have been changed in the new general chapter 621. This adjustment can be applied only for isocratic analysis.

Table 1 Allowed HPLC Adjustments in the Previous General Chapter 621 (official until July 31st, 2014)

Column length	Can be adjusted by as much as ±70 %
Column inner diameter	Can be adjusted if the linear velocity is kept constant.
Particle size	Can be reduced by as much as 50%, but cannot be increased.
Flow rate	Can be adjusted by ±50 %

Table 2 Allowed HPLC Adjustments in the New General Chapter 621 (official after August 1st, 2014)

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 into the range between -25 % to +50 % of the prescribed *L/dp* ratio.
Column inner diameter	Can be adjusted if the linear velocity is kept constant.
Flow rate	Can be adjusted by ±50 %

These changes are based on the theory that resolution can be kept constant as long as *L/dp* is kept constant. For example, if a method using a 150 mm L, 5 µm column (*L/dp* = 150,000 µm /5 µm = 30,000) is transferred to a method using a 50 mm L , 1.6 µm column (*L/dp* = 50,000 µm /1.6 µm = 31,250), similar resolution should be obtained.

Flow rate changes for both a change in column diameter and particle size can be calculated by:

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

where F_1 and F_2 are the flow rates for the original and modified conditions, respectively; dc_1 and dc_2 are the respective column diameters; and dp_1 and dp_2 are the particle sizes.

Materials and Method

Reagents and standards

Reagents: Sulfacetamide, sulfanilamide, timolol maleate, glacial acetic acid, monobasic sodium phosphate, and phosphoric acid were purchased from Sigma-Aldrich. Water was made in house using a Millipore Milli-Q Advantage A10 Ultrapure Water Purification System. Methanol and acetonitrile were purchased from Honeywell.

Standard solutions (Sulfacetamide analysis):
Mobile phase: Methanol, water, and glacial acetic acid (10:89:1).
Diluent: Methanol and 1% glacial acetic acid (10:90).
System suitability solution: 0.2 mg/mL of sulfacetamide and 0.05 mg/mL of sulfanilamide in *Diluent*. Sonicated for 5 min to dissolve.
Standard solution: 0.2 µg/mL of sulfacetamide and 4 µg/mL of sulfanilamide in water.
Standard solutions (Timolol maleate ophthalmic solution analysis):
pH 2.8 phosphate buffer: Dissolved 11.1 g of monobasic sodium phosphate in 1000 mL of water, adjusted with phosphoric acid to a pH of 2.8, filtered and degassed.
Diluent: Prepared a mixture of acetonitrile and *pH 2.8 phosphate buffer* (2:1).
Mobile phase: Prepared a mixture of *pH 2.8 phosphate buffer* and methanol (65:35).
Standard preparation: Transferred 34 mg of timolol maleate to a 25 mL volumetric flask, dissolved in and diluted with water to volume, and mixed. Transferred this stock solution to a 50 mL volumetric flask, added 15 mL of *Diluent*, diluted with water to volume, and mixed.

System

The standard solutions were injected to a Shimadzu Prominence system and Nexera X2 system. The Prominence system consisted of a LC-20AD pump, DGU-20A5R degassing unit, SIL-20AC autosampler, CTO-20AC column oven, SPD-20AV UV-VIS detector equipped with the conventional flowcell, and a CBM-20A system controller. The Nexera X2 system consisted of a LC-30AD pump, DGU-20A5R degassing unit, SIL-30AC autosampler (loop injection mode with 20uL loop), CTO-30A column oven, SPD-M30A photodiode array detector equipped with the standard flow cell, and a CBM-20A system controller.

Results

Adjustment of USP method for speed

A USP method for sulfacetamide, which is a sulfonamide antibiotic, was transferred to a UHPLC method. The USP designates the use of a 4.6 × 150 mm, 5 µm, L1 (ODS) column and a 0.8 mL/min flow rate for the organic impurities analysis of sulfacetamide. A 2.0 × 50 mm, 1.6 µm, L1 column can be used for adjustment of USP method for speed without method revalidation because *L/dp* is kept constant. The new flow rate after method transfer is calculated to 0.47 mL/min according to the equation shown earlier. System suitability solution, standard solution, and sample solution are designated to run in the monograph, but only the chromatogram of the system suitability solution was shown in this poster. The USP method and the ultra-high speed method were run on Prominence and Nexera X2 UHPLC system, respectively. The analytical conditions are shown in Table 3, the analysis results are shown in Fig. 1, and the system suitability results are shown in Table 4. As a result, analysis time and solvent consumption were reduced to 1/10 and 1/15, respectively, with the system suitability requirements met.

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Table 3 Analytical Conditions

Parameter	USP method	Ultra-high speed method
System	: Prominence	Nexera X2
Column	: Shim-pack VP-ODS (150 mm L. x 4.6 mm I.D., 4.6 µm)	Shim-pack XR-ODSIII (50 mm L. x 2.0 mm I.D., 1.6 µm)
Mobile Phase	: See "Reagent and standards"	Same as left
Flow Rate	: 0.8 mL/min	0.47mL/min
Column Temperature	: Ambient	Same as left
Injection Volume	: 10 µL	2 µL
Detection	: UV 254 nm	Same as left

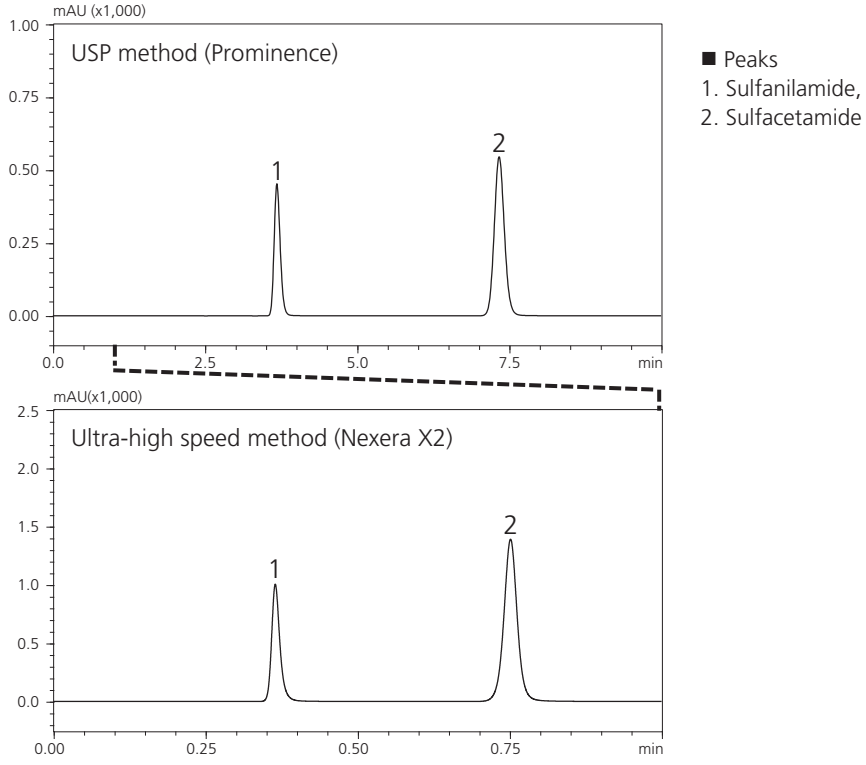

Fig.1 Chromatogram of system suitability solution
(Upper: USP method on Prominence, Lower: Ultra-high speed method on Nexera X2)

Table 4 System suitability results

System suitability requirements		USP method (Prominence)	Ultra-high speed method (Nexera X2)
USP resolution between sulfacetamide and sulfanilamide	≥ 5.0	14.54	12.23
		1.09	1.04
USP tailing factor for sulfacetamide	≤ 1.5	Rt 0.015 %	Rt 0.037 %
Relative standard deviation for sulfacetamide	≤ 2.0 %	Area 0.067 %	Area 0.103 %

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USP method on Nexera X2

A USP method for timolol maleate ophthalmic solution was run on both the Prominence and Nexera X2 UHPLC system without changing the designated method parameters. The USP designates the use of a 4.6 × 150 mm, 5 µm, L1 (ODS) column and a 1.2 mL/min flow rate for the assay. The analytical conditions are shown in Table 5, the analysis results are shown in Fig. 2, and the system suitability results are shown in Table 6. As a result, the system suitability requirements were easily met on the UHPLC system, similar to a standard HPLC system.

Table 5 Analytical Conditions

Parameter	USP method on conventional HPLC	USP method on UHPLC
System	: Prominence	Nexera X2
Column	: Shim-pack VP-ODS (150 mm L. x 4.6 mm I.D., 4.6 µm)	Same as left
Mobile Phase	: See "Reagent and standards"	Same as left
Flow Rate	: 1.2 mL/min	Same as left
Column Temperature	: 40 °C	Same as left
Injection Volume	: 10 µL	Same as left
Detection	: UV 295 nm	Same as left

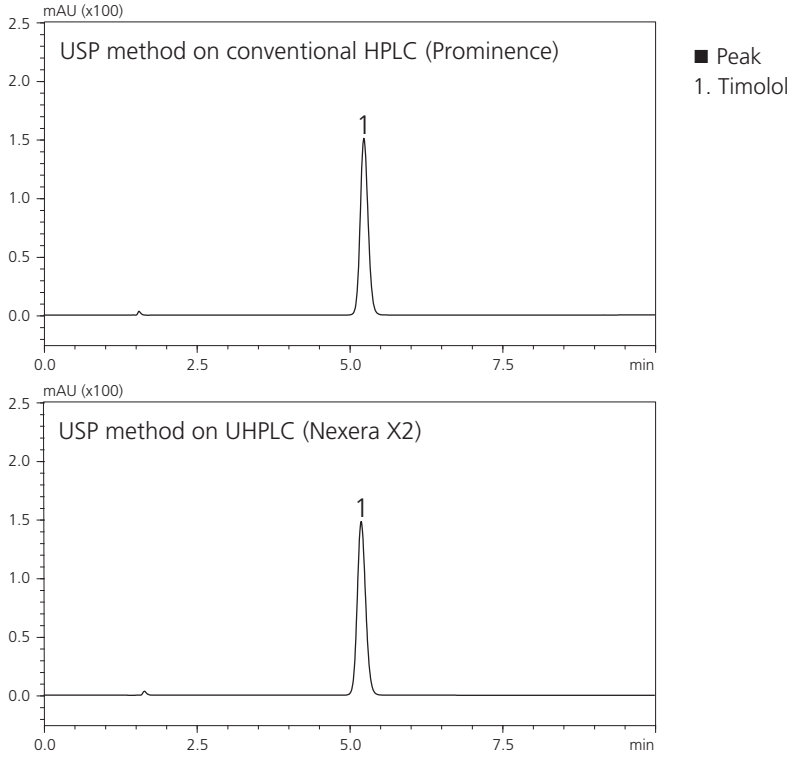

Fig.2 Chromatogram of a standard preparation
(Upper: USP method on Prominence, Lower: USP method on Nexera X2)

Table 6 System suitability results

System suitability requirements		USP method (Prominence)	USP method (Nexera X2)
USP tailing factor	≤ 2.0	1.12	1.11
		6354	6965
USP column efficiency	≥ 3600	Rt 0.027 %	Rt 0.082 %
Relative standard deviation	≤ 2.0 %	Area 0.034 %	Area 0.062 %

Conclusions

A USP method was successfully transferred to an ultra-high speed method with the system suitability requirements met. As a result, analysis time and solvent consumption were reduced to 1/10 and 1/15, respectively. Additionally, a USP method was run on the Nexera X2 UHPLC system without changing method parameters. The system suitability requirements were easily met on the UHPLC system, similar to a standard HPLC system. This result means that this system is also suitable for those who are running traditional USP methods on a standard HPLC system and considering the adoption of a UHPLC system for high speed analysis of USP methods in the future.

Reference

USP General Chapter 621, USP 37-NF 32, First supplement
USP Monograph, Sulfacetamide, USP 37-NF 32, First supplement
USP Monograph, Timolol maleate ophthalmic solution, USP 37-NF 32, First supplement

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