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Application News

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High Performance Liquid Chromatography

High-Speed Analysis of Pramipexole following the Draft Guidance of International Harmonization of Pharmacopoeias

In recent years, ultra-high performance liquid chromatography (hereinafter, UHPLC) has been widely adopted in the pharmaceutical field to improve efficiency and productivity in analytical work. Responding to these situations, General Chapter <621> CHROMATOGRAPHY in the 40th Edition of the United States Pharmacopeia (USP) and "Adjustment of chromatographic condition" in the 8th Edition of the European Pharmacopeia (EP) allow changes in high speed analysis conditions within a range that conforms to the system suitability test. On the other hand, the Japanese Pharmacopeia (JP) does not specify a clear allowable range for changes in analytical conditions, and as of this writing (March 2018), both USP and EP essentially do not allow high-speed gradient separation.

In this situation, the Japanese, US and European Pharmacopeias are working toward international harmonization, and there is a movement to unify the allowable ranges of analytical conditions. In this movement, the new allowable range of gradient separation is also expected to be specified. (1)

This article introduces an application of a high speed analysis of pramipexole dihydrochloride analogs,(2) as described in the 40th Edition of USP, using Shimadzu integrated LC system Nexera™-i MT based on the draft guidance of international harmonization of JP/USP/EP. It should be noted that this draft is based on the draft version of the international harmonization guidelines for public comment published in July 2017, which may differ from the final version.

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Allowable Range of Changes in Gradient Elution

The allowable ranges of analytical conditions for gradient elution are specified in detail in the international harmonization draft.(1) Only the principal items are excerpted here.

Column dimensions: The ratio of the column length (L) and the particle size (dp) L/dp shall be within the range of -25 % to +50 %. Columns for superficially porous particles are specified separately.

Flow rate: Adjustment of the flow rate is necessary when the particle size is changed. The adjusted flow rate shall conform to the following equation.

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

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 of the column indicated in the monograph (µm)

dp_2 : particle size of the column used (µm)

When the particle size is changed from less than 3 µm to 3 µm or more, an additional increase in the linear velocity is allowed, when the column efficiency does not drop by 20 % or more.

Gradient time: The gradient volume changes in proportion to the column volume. Since the gradient volume is the product of the gradient time (t_G) and the flow rate (F), the following formula is used to maintain a constant ratio of the gradient volume to the column volume.

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

t_{G1}: original gradient time

t_{G2}: adjusted gradient time

High-Speed Pramipexole Hydrochloride Analog Test

Pramipexole is a drug that is used for the treatment of Parkinson's disease. The pharmacopeias specified gradient elution as a test method and required revalidation when a high-speed analysis was applied. Anticipating the possibility that changes in the analytical conditions related to high-speed analysis will be allowed for gradient elution in the draft guidance of international harmonization, as mentioned above, here, high-speed analysis was employed based on conditions conforming to the draft for public comment of July 2017. For transfer of analytical conditions, the dedicated Method Transfer tool for Nexera™-i MT contained in Shimadzu's workstation software LabSolutions™ (hereinafter, LabSolutions)*1 was used. With Method Transfer, the gradient profile and flow rate are read from the existing method in LabSolutions, and the gradient time is transferred simply by inputting the column dimensions and flow rate to be used. Since the result can be registered directly in a method file in LabSolutions, it is possible to avoid transcription errors due to manual work. Fig. 1 shows the Method Transfer screen. Table 1 shows the original USP conditions and the conditions after transfer to high-speed analysis (UHPLC), and Tables 2 and 3 show the detailed analytical conditions.

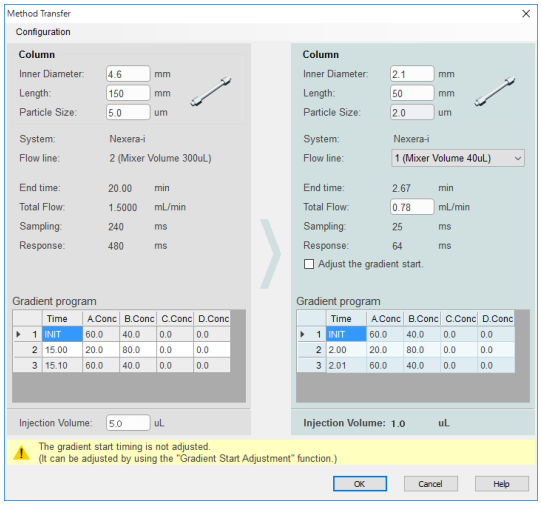

Fig. 1 Dedicated Method Transfer Screen for Nexera™-i MT

*1 The version as of January 2018 includes a calculation algorithm which is generally similar but does not conform completely to the international harmonization draft.

Table 1 High-Speed Analytical Conditions Based on International Harmonization Draft

	USP	Transfer (UHPLC)	note
Column length (*L*) (mm)	150	50	User choice
Column diameter (*dc*) (mm)	4.6	2.1	User choice
Particle size (*dp*) (µm)	5.0	2.0	User choice
L/dp	30.0	25.0	-17 %
Flow rate (mL/min)	1.5	0.78	(A)
Gradient factor		0.13	(B)
Gradient			
%B	Time (min)	Time (min)	
40	0	0	
80	15	2.00	
40	15.1	2.01	
40	20	3.5	*2
Injection Volume (µL)	5	1	*3

*2 Completion time is adjusted for the dwell volume.
*3 The injection volume is different from the international harmonization draft, and is reduced in proportion to the cross-sectional area of the column.

Table 2 Analytical Conditions Conforming to USP

Column	: Shim-pack™ VP-ODS
	: 150 mmL. × 4.6 mmI.D., 5.0 µm
Mobile phase	: A) 67 mmol/L (Potassium) Phosphate Buffer (pH 3.0) Containing 21 mmol/L 1-Octanesulfonic Acid Sodium Salt
	B) Solution A / Acetonitrile (1/1)
Flow rate	: 1.5 mL/min
Time program	: B Conc. 40 % (0 min) → 80 % (15 min) → 40 % (15.1 - 20 min)
Column temp.	: 40 °C
Injection volume	: 5 µL
Detection	: UV 264 nm
Sample	: Pramipexole Dihydrochloride

Table 3 Analytical Conditions for UHPLC

Column	: Shim-pack™ GIST C18
	: 50 mmL. × 2.1 mmI.D., 2.0 µm
Mobile phase	: A) 67 mmol/L (Potassium) Phosphate Buffer (pH 3.0) Containing 21 mmol/L 1-Octanesulfonic Acid Sodium Salt
	B) Solution A / Acetonitrile (1/1)
Flow rate	: 0.78 mL/min
Time program	: B Conc. 40 % (0 min) → 80 % (2.00 min) → 40 % (2.01 - 3.5 min)
Column temp.	: 40 °C
Injection volume	: 1 µL
Detection	: UV 264 nm
Sample	: Pramipexole Dihydrochloride

Analysis Results

Fig. 2 shows the results of USP-compliant and UHPLC analyses of pramipexole dihydrochloride, and Fig. 3 shows enlarged chromatograms in the vicinity of elution of pramipexole dihydrochloride. Table 4 shows the results of a system suitability test. Both USP-compliant and UHPLC results meet criteria of all test items.

The results presented above showed that smooth method transfer is possible using the dedicated Method Transfer tool for Nexera™-i MT. As the pharmacopeias are expected to allow method changes in gradient analysis, in the future, it appears that use of tools like Method Transfer will also become even more important for enhancing ease-of-work.

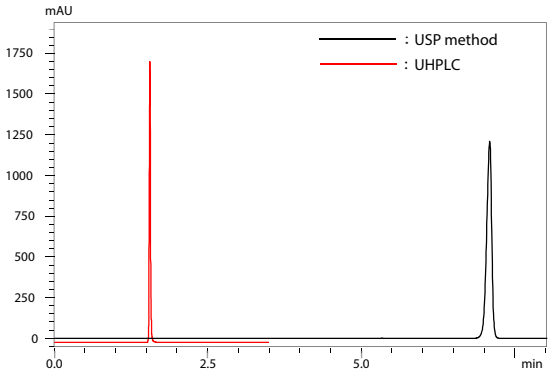

Fig. 2 Results of Analysis of Pramipexole Dihydrochloride

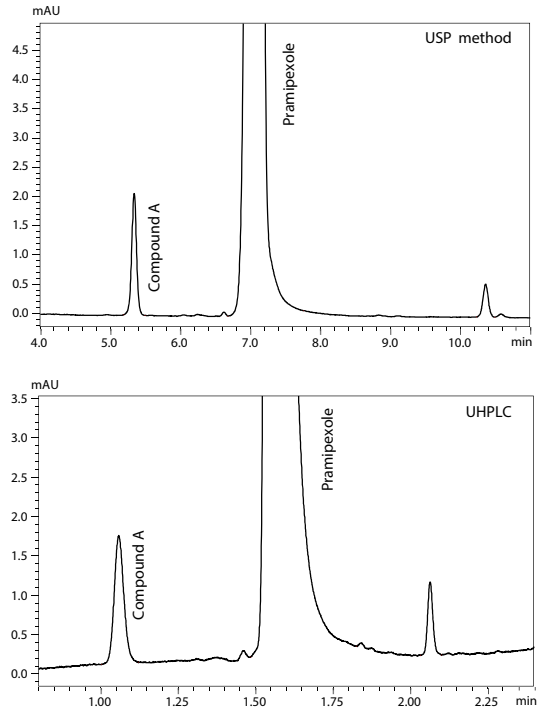

Fig. 3 Enlarged Chromatographs of Vicinity of Pramipexole Dihydrochloride

Table 4 Results of System Suitability Test

Test item	Criteria	USP	UHPLC	Judgement
Resolution (between compound A and pramipexole)	≥6.0	12.3	10.7	PASSED
Tailing factor	≤2.0	0.83	1.10	PASSED
Relative standard deviation	≤5.0 %	0.161	0.265	PASSED

[References]

(1) International Harmonization (Stage 4), Pharmaceuticals and Medical Devices Agency
http://www.pmda.go.jp/english/rs-sb-std/standards-development/jp/0004.html
(2) USP pharmacopoeia 40: 5795 "Pramipexole Dihydrochloride"

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