SHIMADZU
Excellence in Science

Application News

No. L542

High Performance Liquid Chromatography

High-Speed Analysis of Linezolid following the Draft Guidance of International Harmonization of Pharmacopoeias

Linezolid is a new class of synthetic antibiotic with an oxazolidinone skeleton (Fig. 1). It was approved as a therapeutic agent in the United States in 2000 and in Japan in 2001. Linezolid was also listed in the Second Supplement to the 40th Edition of the United States Pharmacopeia (USP) published in 2017.

On the other hand, in international harmonization of the respective pharmacopeias, which is currently under study, the allowable range of changes in analytical conditions is specified in the draft guidance of international harmonization of JP/USP/EP [(1)]. Sharing a common allowable range of changes in high speed analysis conditions by all countries is important for achieving higher efficiency in drug development.

This article introduces an example of analysis of linezolid based on the USP and an example of its high-speed analysis based on the draft guidance of international harmonization of JP/USP/EP using Shimadzu Nexera™ Series and Shim-pack Scepter™ C18. It should be noted that this draft is based on the draft version published when public comment was solicited in July 2017 and may differ from the content which is finally adopted.

M. Oshiro

Outline of Shim-pack Scepter

Column selection is critical when high-speed analysis is applied. The separation performance of a column is proportional to the column length, but is inversely proportional to the particle size of the packing material. In other words, when the column size is shortened further while maintaining the same separation performance, smaller particles size must be used at the same time. When making these changes, it is possible to realize higher analysis speed with the same separation paturm by using the same packing material functional group and its modification condition.

Because the Shim-pack Scepter Series uses common packing materials with a wide range of particle sizes (1.9, 3, 5 µm) and also offers an extensive lineup of column size, it is possible to use the Scepter Series in applications from high-speed analysis to preparative analysis. As a result, seamless analysis method transfer is possible.

In this Application News, high-speed analysis using the column with 3 µm and 1.9 µm of particle size was examined.

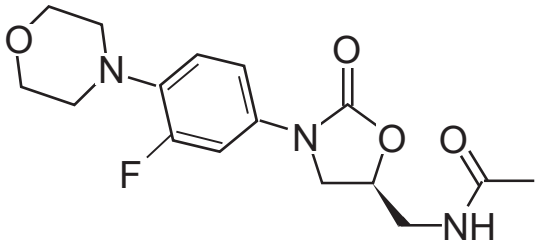

Fig. 1 Linezolid

High-Speed Analysis Conditions

As introduced in Application News L524, harmonization activities for method and pharmaceutical additive monographs in the Japanese Pharmacopoeia (JP), European Pharmacopoeia (EP), and United States Pharmacopeia (USP) are underway in the Pharmacopeial Discussion Group, and the draft guidance of international harmonization of JP/USP/EP on chromatography was published when public comment was solicited in 2017. This draft specifies the allowable range of changes in analytical test conditions, which is not included in the existing JP. Therefore, high-speed analysis may also become possible in the JP in the future.

Table 1 shows the USP analysis conditions used in the quantitative analysis method (Assay) for linezolid, together with the high-speed analysis conditions (UHPLC: ultra-high performance liquid chromatography) that were set based on the international harmonization draft. Fig. 2 shows the allowable range for adjustment of the analysis conditions for chromatography (gradient elution) in the draft used in setting the high-speed conditions. Only the principal items are excerpted here.

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

	USP	UHPLC	Note
Column length (*L*) (mm)	75	50	User choice
Column diameter (*dc*) (mm)	4.6	2	User choice
Particle size (*dp*) (µm)	3	1.9	User choice
L/dp	25	26.3	-5.20 %
Flow rate (mL/min)	1.5	0.45	
Gradient factor		0.42	
Gradient (%B)	Time (min)	Time (min)	
20	0	0	
43	8	3.4	
100	18	7.6	
100	25	10.6	
20	25.01	10.61	
20	30	12.7	

Column dimensions: The ratio *L/dp* of the column length (*L*) and the particle size (*dp*) of the column to be changed 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 also necessary when the particle size is changed. The adjusted flow rate shall conform to the following equation.

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

F_1 : flow rate before change (mL/min)
F_2 : flow rate after change (mL/min)
dc_1 : internal diameter of column before change (mm)
dc_2 : internal diameter of column after change (mm)
dp_1 : particle size of column before change (µm)
dp_2 : particle size of column after change (µm)

When the particle size is to be changed from 3 µm or more to less than 3 µm, an increase in the linear velocity is allowed within the range where column efficiency does not decrease by 20 % or more.

Gradient time: The gradient volume changes in proportion to the column volume. The change is calculated using the following equation.

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

t_{G1} : gradient time before change
t_{G2} : gradient time after change

Fig. 2 Allowable Range for Adjustment of Analysis Conditions for Chromatography in International Harmonization Draft (Gradient Elution, Principal Items)

Evaluation of High-Speed Analysis Conditions

Fig. 3 shows the results of USP and UHPLC analyses of a linezolid solution (0.8 mg/L). Fig. 4 shows the enlarged chromatograms under these two conditions. Compound D was identified from the relative retention time to linezolid. Table 2 and Table 3 show USP analysis conditions and UHPLC analysis conditions respectively. Table 4 shows the results of an evaluation of a system suitability test based on the results of this study. Both the USP results and the UHPLC results satisfied the system suitability requirements.

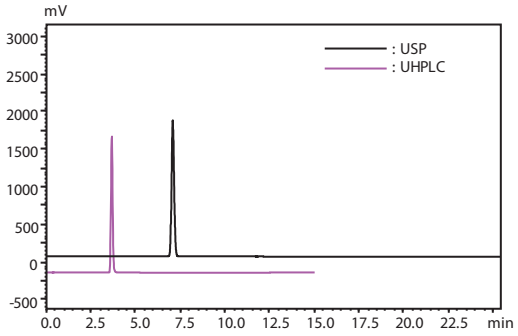

Fig. 3 Chromatograms of Linezolid

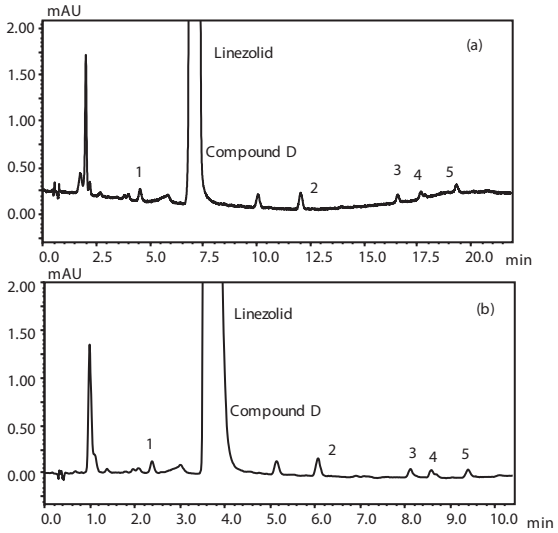

Fig. 4 Enlarged Chromatograms of Linezolid (a) USP, (b) UHPLC

Table 2 USP Analysis Conditions

Column	: Shim-pack Scepter C18 (75 mmL×4.6 mmI.D., 5 µm)
Mobile phase	: A) Methanol/Acetonitrile/1.4 g/L Monobasic potassium phosphate = 15/5/80 (v/v) B) Methanol/1.4 g/L Monobasic potassium phosphate = 50/50 (v/v)
Flow rate	: 1.5 mL/min
Time program	: B Conc.20 % (0 min) →43 % (8 min) →100 % (18 min) →100 % (25 min) →20 % (25.01-30 min)
Injection volume	: 10 µL
Column temp.	: 30 °C
Detection	: UV 254 nm

Table 3 UHPLC Analysis Conditions

Column	: Shim-pack Scepter C18 (50 mmL×2.0 mmI.D., 1.9 µm)
Mobile phase	: A) Methanol/Acetonitrile/1.4 g/L Monobasic potassium phosphate = 15/5/80 (v/v) B) Methanol/1.4 g/L Monobasic potassium phosphate = 50/50 (v/v)
Flow rate	: 0.45 mL/min
Time program	: B Conc.20 % (0 min) →43 % (3.4 min) → 100 % (7.6 min) →100 % (10.6 min) → 20 % (10.61-12.7 min)
Injection volume	: 2 µL
Column temp.	: 30 °C
Detection	: UV 254 nm

Table 4 Results of System Suitability Test

System suitability requirement		USP	UHPLC	Judgement
Resolution (between linezolid and compound D)	≥3.0	12	7.7	PASSED
Tailing factor	≤1.5	1.1	1.2	PASSED
Relative standard deviation	≤1.0 %	0.1	0.07	PASSED

Table 5 shows the relative retention times of impurities to linezolid used in this analysis under the USP and UHPLC conditions. It is possible to study high-speed analysis while maintaining the separation paturm more simply by using the Shim-pack Scepter Series.

Table 5 Comparison of Relative Retention Times for USP and UHPLC Conditions

Peak	Relative retention times to linezolid	
	USP	UHPLC
1	0.6	0.7
Compound D	1.4	1.4
2	1.7	1.7
3	2.3	2.2
4	2.5	2.3
5	2.7	2.6

Conclusion

An analysis of linezolid was conducted using the Shimadzu Nexera Series and Shim-pack Scepter C18. When high-speed analysis was used based on the USP and the international harmonization draft, both conditions satisfied the system suitability test. High-speed analysis while maintaining the separation paturm is possible more simply by using Shim-pack Scepter C18 in the analysis.

[Reference]
(1) International Harmonization (Stage 4), Pharmaceuticals and Medical Devices Agency.

First Edition: Jul. 2019

SHIMADZU

Shimadzu Corporation

www.shimadzu.com/an/