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Application News

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High Performance Liquid Chromatography

High Speed Analysis of Pharmaceutical Impurities in Compliance with European Pharmacopoeia Using Nexera-i MT

In recent years, the development of short-time analytical methods for improving analytical task efficiency and productivity is promoting the uptake of an ultra-high-speed analytical technology that uses UHPLC systems and columns packed with microparticles in research and development departments in the pharmaceutical field. This trend also applies to pharmacopoeia. For example, according to "Adjustment of chromatographic condition"[*1] described in the 8th edition of the European Pharmacopoeia (EP), adjustments to parameters in TLC, LC, GC and SFC are only allowed when the system suitability requirements are satisfied. In such a case, revalidation is not required.

This article introduces an example of high speed analysis of pharmaceuticals and related substances in compliance with the EP using the Nexera-i MT integrated high performance liquid chromatograph.

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Allowable Adjustment Range of HPLC

The LC section in "Adjustment of chromatographic condition" is broadly classified into isocratic elution and gradient elution.

For gradient elution, the allowable adjustment range of methods differs from that of isocratic elution because peak-shifting caused by unstable gradient profile of the mobile phase can lead to misidentification and overlapping of multiple peaks. For example, in terms of column particle size, while a reduction of up to 50% is possible for isocratic elution, particle size cannot be adjusted for gradient elution. Furthermore, in the case of gradient elution, it is stated that the elution time of the principal peak must be within ±15 % of that in the testing method. Thus, the adjustments of many parameters are restricted for gradient elution and further high speed analysis is practically impossible. Therefore high speed analysis can only be achieved for isocratic elution.

High Speed Analysis of Ivermectin and Related Substances

Ivermectin, belonging to macrolides, is known as a therapeutic drug for strongyloidiasis, an antiscabietic and an antiparasitic agent for animals. The two main components of ivermectin are H_2B_{1a} (molecular weight: 875) and H_2B_{1b} (molecular weight: 861). The former makes up more than 90 % of its composition.

Table 1 Analytical Conditions

System	:	Nexera-i MT
Column 1 (Conventional)	:	Shim-pack GIST C18 (250 mm L, 4.6 mm I.D., 5 µm)
Flow rate 1	:	1.0 mL/min
Column 2 (High speed)	:	Shim-pack GIST C18 (150 mm L, 4.6 mm I.D., 3 µm)
Flow rate 2	:	1.5 mL/min
Mobile phase	:	A) Water B) Methanol C) Acetonitrile A/B/C=15/34/51 (v/v/v)
Column temp.	:	25 °C
Injection volume	:	20 µL
Detection	:	UV254 nm

In this research we examined reducing the analysis time within the adjustment range allowed by the EP. Table 1 lists the analytical conditions that comply with both the ivermectin related substances testing section[*2] and the allowable adjustment range assigned in the EP. Since the Nexera-i MT used in analysis features both HPLC and UHPLC flow lines, it allows migration between conventional analysis and high speed analysis within a single system. The Shim-pack GIST C18 series was used for the analytical columns. The analytical conditions other than the analytical columns and flow rate are the same as those listed in the EP.

Fig. 1 shows resulting chromatograms of ivermectin standard solution (0.8 mg/mL). The high speed analysis provided approximately 60 % and 40 % reductions of analysis time and mobile phase consumption respectively while maintaining enough separation. Table 2 shows the results of system suitability test. Both conventional analysis and high speed analysis passed the test.

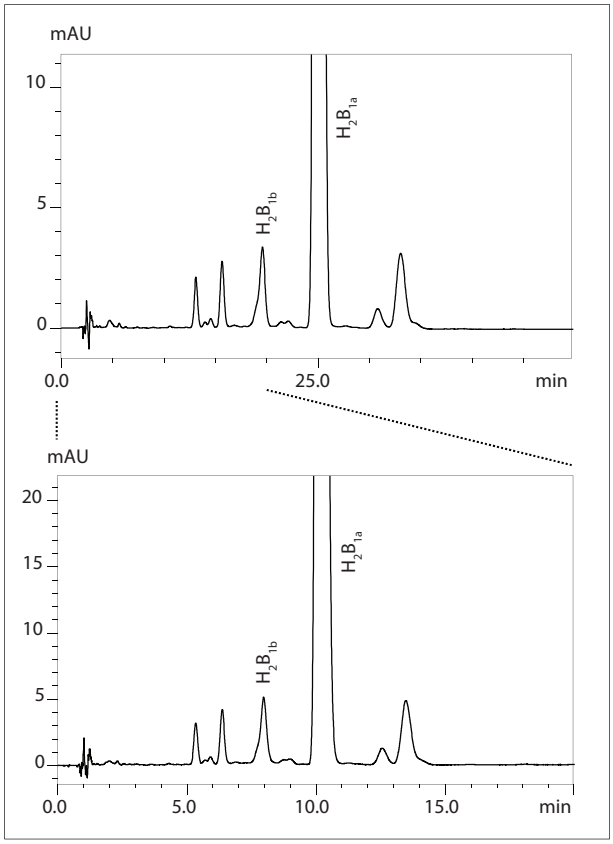

Fig. 1 Chromatograms of Ivermectin Standard Solution
Upper: Conventional Analysis Using HPLC Flow Line (Column 1)
Lower: High Speed Analysis Using UHPLC Flow Line (Column 2)

Table 2 Results of System Suitability Test

System suitability requirements		Results		Judgements
		Conventional	High speed	
Resolution (H_2B_{1b} and H_2B_{1a})	≥ 3.0	5.1	4.7	PASS
Signal-to-noise ratio (0.4 µg/mL)	≥ 10	40	38	PASS
Symmetry factor	≤ 2.5	1.1	1.2	PASS

High Speed Analysis of Diclofenac Sodium and Related Substances

Diclofenac is widely used as an antipyretic and a pain-reliever. Here we introduce an example of high speed analysis of a diclofenac sodium and related substances based on the EP.

Fig. 2 shows the resulting chromatograms of diclofenac standard solution (1.0 mg/mL). Table 3 lists the analysis conditions that comply with both the testing section[*3] of diclofenac sodium related substances and the allowable adjustment range assigned in the EP. The analytical columns used in conventional analysis and high speed analysis were both the same as those used in the analysis of ivermectin. A commercially-available reagent for system suitability testing was used as the reference standard.

In conventional analysis, the mobile phase flow rate assigned in the EP is 1.0 mL/min. Despite adjusting the flow rate to 0.8 mL/min in this research due to the column pressure tolerance, which is within the allowable adjustment range, the obtained results meet the system suitability requirements (Table 4). High speed analysis also passed the system suitability test. The high speed analysis provided approximately 70 % and 40 % reductions of analysis time and mobile phase consumption respectively while maintaining enough separation.

As demonstrated above, Nexera-i MT not only facilitated migration from conventional analysis to high speed analysis but also provided results of an equal level.

Table 3 Analytical Conditions

System	:	Nexera-i MT
Column 1 (Conventional)	:	Shim-pack GIST C18 (250 mm L, 4.6 mm I.D., 5 µm)
Flow rate 1	:	0.8 mL/min
Column 2 (High speed)	:	Shim-pack GIST C18 (150 mm L, 4.6 mm I.D., 3 µm)
Flow rate 2	:	1.4 mL/min
Mobile phase	:	A) Sodium phosphate buffer (pH 2.5) B) Methanol A/B=34/66 (v/v)
Column temp.	:	25 °C
Injection volume	:	20 µL
Detection	:	UV 254 nm

Table 4 Results of System Suitability Test

System suitability requirement		Results		Judgement
		Conventional	High speed	
Resolution (impurity F and Diclofenac)	≥ 4.0	6.8	5.1	PASS

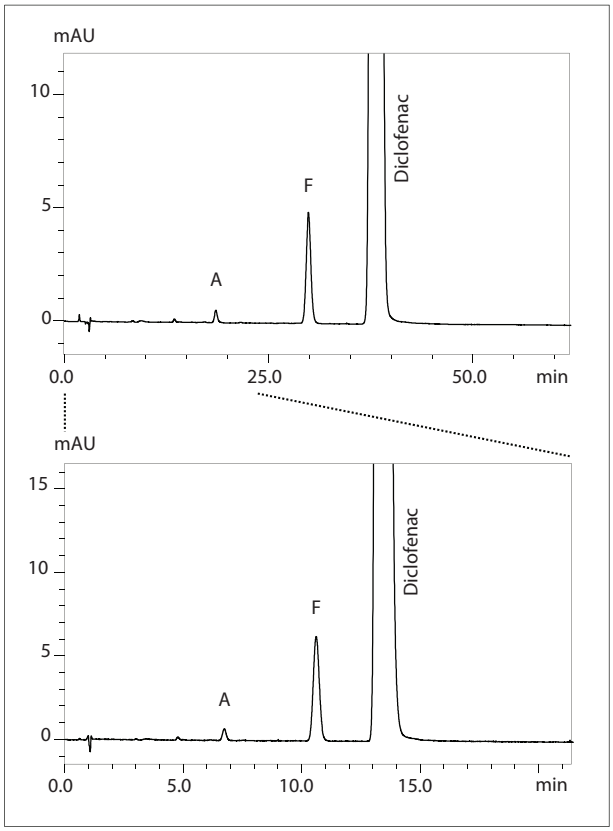

Fig. 2 Chromatograms of Diclofenac Standard Solution
Upper: Conventional Analysis Using HPLC Flow Line (Column 1)
Lower: High Speed Analysis Using UHPLC Flow Line (Column 2)

<References>
*1 European Pharmacopoeia 8.0, 04/2009:20246
2.2.46. Chromatographic separation techniques
*2 European Pharmacopoeia 8.8, 04/2016:1336 "Ivermectin"
*3 European Pharmacopoeia 8.8, 07/2014:1002 "Diclofenac sodium"

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