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Application News

Nexera™ High Performance Liquid Chromatograph Dual Injection System

Simultaneous Analysis for Drug Purity Test and Quantitative Assay

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User Benefits

- Two different analyses, such as purity tests and quantitative assays described in the pharmacopoeia, can be performed simultaneously on a single HPLC to increase the throughput of analytical work.
- Creating a high speed analytical method that meets the system suitability prescribed in the monograph by modifying analytical conditions within the allowances described in USP <621> provides time reduction and solvent saving in HPLC analysis conforming to USP.

■ Introduction

The United States Pharmacopeia (USP) and the Japanese Pharmacopoeia (JP) prescribe analyses with a variety of protocols, such as purity tests and quantitative assays, to ensure the quality of drug products. When such pharmacopoeias specify HPLC analyses, it often suggests multiple tests with different conditions depending on the purpose of the analyses, to be performed separately with multiple HPLCs.

In this article, The high speed analytical methods for both purity test (related substances) and the quantitative assay of ibuprofen described in "USP43-NF38" were created conforming to USP General Chapter <621> Chromatography (hereafter, referred to as "USP <621>"), describing allowances for modifying analytical conditions. Then the high speed purity test and the high speed quantitative assay were executed with the Nexera Dual Injection System, simultaneous.

■ Nexera Dual Injection System Capable of Simultaneous Two-Line Analyses

The Nexera Dual Injection System has two independent flow paths, enabling the simultaneous execution of two analyses with respective different conditions by injecting a common sample solution into each path. Fig. 1 shows the flow path diagram.

In this study, Line ① was used for purity test, and Line ② for the quantitative assay of ibuprofen.

Fig. 2 shows the appearance of the system employed. This system enables simultaneous two-line analyses in the same footprint as that of a single line system.

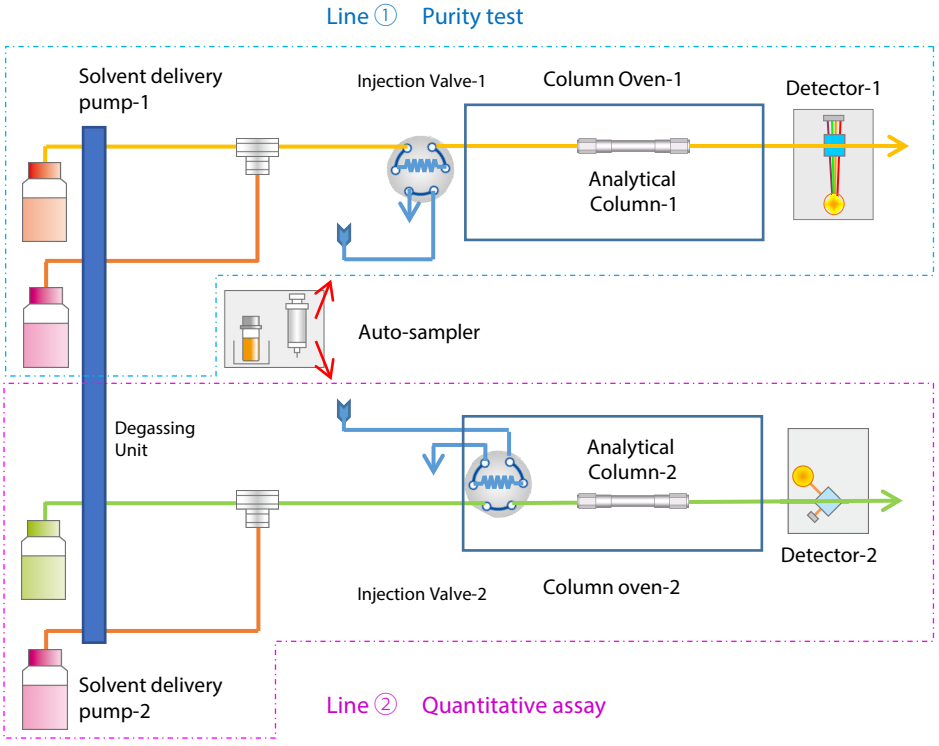

Fig. 1 Flow Path Diagram of Nexera Dual Injection System

Fig. 2 Nexera Dual Injection System (External View)

■ High Speed Purity Test of Ibuprofen

The purity test of ibuprofen specified in "USP43-NF38" was conducted in Line ①.

The analytical conditions were adjusted as allowed in USP <621> to reduce the analysis time.

Fig. 3 shows the chromatogram of the system suitability check sample containing ibuprofen and valerophenone (internal standard). Table 1 and Table 2 show the analytical conditions and the results of the system suitability test, respectively. It was confirmed that the high speed ibuprofen purity test method modified within the allowances prescribed in USP <621> met the requirements of the system suitability for resolution.

Table 1 Analytical Conditions (Purity Test)

Column	: Shim-pack Velox™ C18 (150 mm × 3 mm I.D., 2.7 µm)*1
Flow Rate	: 1.8 mL/min
Mobile Phase	: A; 10 mmol/L phosphoric Acid Water (pH 2.5) B; Acetonitrile A / B = 33 : 67
Column Temp.	: 35 ˚C
Injection vol.	: 1.0 µL
Vial	: SHIMADZU LabTotal™ for LC 1.5 mL, Glass*2
Detection	: SPD-M40 at 214 nm

*1 P/N: 227- 32010-04
*2 P/N: 227-34001-01

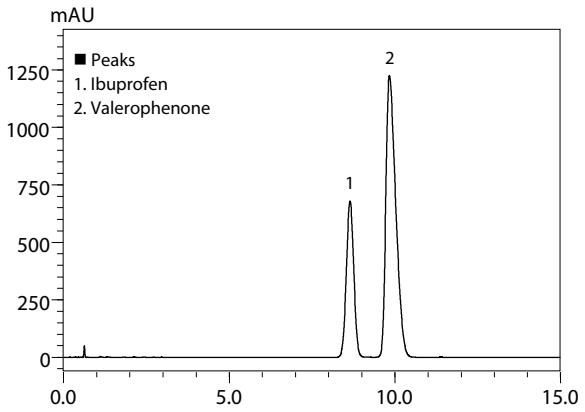

Fig. 3 Chromatogram of System Suitability Check Sample for Purity Test

Table 2 Results of System Suitability for Purity Test

System suitability items		Criteria	Results
Relative retention time	Valerophenone (When the Ibuprofen retention time is 1.0)	0.8	0.9
Resolution	Valerophenone and Ibuprofen	≥ 2.0	9.1

■ High Speed Quantitative Assay of Ibuprofen

The quantitative assay of ibuprofen specified in "USP43-NF38" was conducted in Line ②.

As in the purity test, the analytical conditions were adjusted as allowed in USP <621> to reduce the analysis time.

Fig. 4 shows the chromatogram of a mixture containing ibuprofen, its degradation product 4-isobutylacetophenone (referred to as Ibuprofen Related Compound C in the USP monograph), and valerophenone (internal standard). Table 3 and Table 4 show the analytical conditions and the results of the system suitability test, respectively. It was confirmed that the high speed ibuprofen quantitative assay method modified within the allowances prescribed in USP <621> met the requirements of the system suitability for resolution.

Table 3 Analytical Conditions (Quantitative Assay)

Column	: Shim-pack™ GIST C18-HP (75 mm × 3 mm I.D., 3 µm) *3
Flow Rate	: 1.0 mL/min
Mobile Phase	: A; 1 %w/v Chloroacetic acid water (pH 3.0 adjusted with ammonium hydroxide) B; Acetonitrile A / B = 1 : 1
Column Temp.	: 35 ˚C
Injection vol.	: 1.0 µL
Vial	: SHIMADZU LabTotal for LC 1.5 mL, Glass
Detection	: SPD-40 at 254 nm

*3 P/N: 227- 30040-03

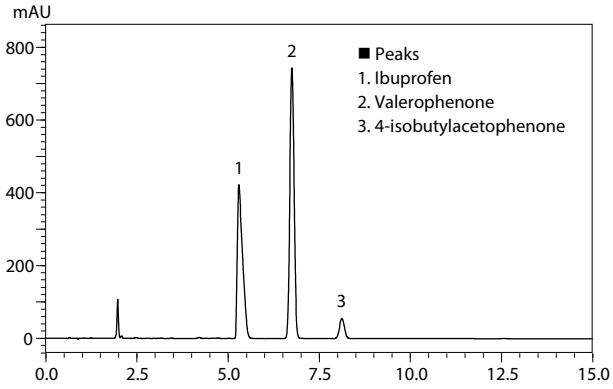

Fig. 4 Chromatogram of System Suitability Check Sample for Quantitative Test

Table 4 Results of System Suitability for Quantitative Assay

System suitability items		Criteria	Results
Relative retention time	Valerophenone (When the Ibuprofen retention time is 1.0)	1.4	1.3
	4-isobutylacetophenone (When the Valerophenone retention time is 1.0)	1.2	1.2
Resolution	Ibuprofen and Valerophenone	≥ 2.5	5.8
	Valerophenone and 4-isobutylacetophenone	≥ 2.5	5.8
Symmetry factor	Ibuprofen	≤ 2.5	2.0
	Valerophenone	≤ 2.5	1.0
	Valerophenone and 4-isobutylacetophenone	≤ 2.5	1.0

■ Simultaneous Analysis for Purity Test and Quantitative Assay

Simultaneous six times repeated analyses for the purity test and the quantitative assay were executed with the Nexera Dual Injection System.

Fig. 5 shows the chromatograms from the purity test, and Table 5 shows the relative standard deviations of the retention time and peak area. Although purity tests do not require the system suitability for repeatability, good repeatability was obtained.

Similarly, Fig. 6 shows the chromatograms from the quantitative test, and Table 6 shows the relative standard deviations of the retention time and peak area as the system suitability test for repeatability. Good repeatabilities that meet the system suitability criterion were obtained.

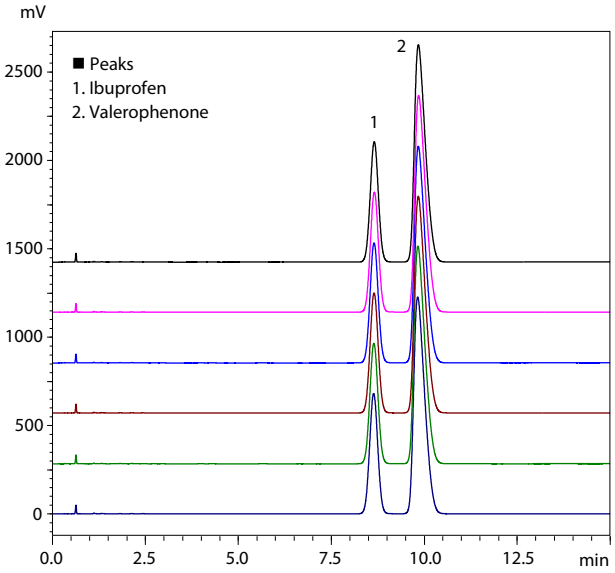

Fig. 5 Chromatograms from Simultaneous Analysis (Purity Test)

Table 5 Repeatability of Simultaneous Analysis (Purity Test)

Items		Relative standard deviation (%) n=6
Ibuprofen	Retention time	0.10
	Peak area	0.18
Valerophenone	Retention time	0.08
	Peak area	0.18

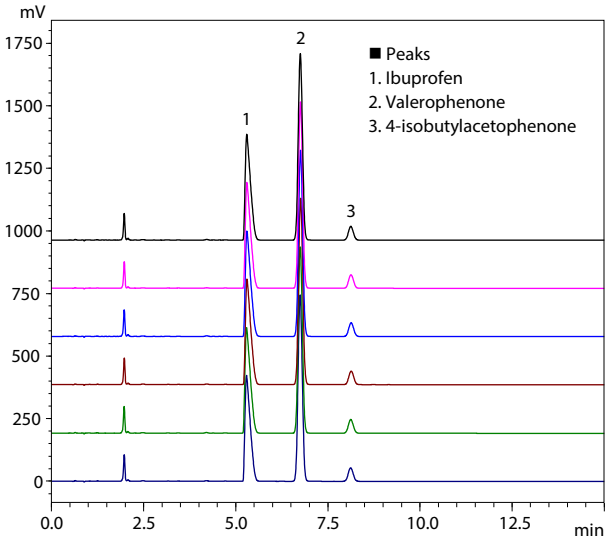

Fig. 6 Chromatograms from Simultaneous Analysis (Quantitative Test)

Table 6 Results of System Suitability Items for Quantitative Assays

System suitability items		Relative standard deviation (%) n=6	
		Criteria	Results
Ibuprofen	Retention time	≤ 2.0	0.09
	Peak area	≤ 2.0	0.05
Valerophenone	Retention time	≤ 2.0	0.07
	Peak area	≤ 2.0	0.07
4-isobutylacetophenone	Retention time	≤ 2.0	0.07
	Peak area	≤ 2.0	0.07

■ Conclusion

Adjusting the analytical conditions of both the purity test (related substances) and quantitative assay for ibuprofen specified in "USP43-NF38" according to USP <621> enabled high speed analyses that meet the requirements of the respective system suitability.

Furthermore, the analyses for the purity test and quantitative assay of ibuprofen were simultaneously performed under the modified conditions for high speed analyses using the Nexera Dual Injection System, resulting in good resolution and repeatability.

Combining high speed analytical conditions and simultaneous analysis can significantly reduce the analysis time.

Although this article introduced simultaneous analyses related to the pharmacopoeia, this procedure can be easily applied to other HPLC applications for multiple target components in the food and/or environmental industries, etc., and will improve the throughput of routine analyses.

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