SHIMADZU
Excellence in Science

Application News

No. HPLC-009

High Performance Liquid Chromatography

Ultra-high Speed Analysis of Ibuprofen within USP <621> Allowed Limits by Nexera Method Scouting

In recent years, high-throughput analytical techniques have been developed for effective analysis and productivity improvement. For HPLC, analysis using small particle columns has been attracting increased attention. In the recent revisions of USP <621> and EP <2.2.46>, the modification of parameters is allowed only when the chromatogram improvement is still within the stated system suitability factors.

This application note shows an example of Ibuprofen analysis from the USP-NF. This analysis was based on USP <621> and done by using the Nexera Method Scouting System, with a conventional column, Shim-pack VP-ODS, and a core-shell column, Kinetex XB-C18.

■Allowed HPLC Adjustment

Table 1 shows the allowed deviations of LC parameters per USP <621> and EP <2.2.46>. The specific allowed deviations include column length, particle size, and flow rate. Within these allowed limits, the change of method is only regarded as an adjustment of the method, so there is no need for method revalidation after modifications.

Many users in the pharmaceutical field examine the possibility of method transfer by using the allowed adjustments in the USP/EP method as a way to speed up new drug development and reduce solvent and waste disposal cost.

Table 1: Allowed HPLC Adjustment of USP <621> and EP <2.2.46>

	USP General Chapter <621>	EP General Chapter <2.2.46>
Column Length	±70%	±70%
Column I.D.	No limit, but keep constant linear velocity	±25%
Particle Size	-50%	-50%
Flow Rate	±50%	±50%
Temperature	±10℃	±10℃ Maximum 60℃
Injection Volume	Acceptable as long as it satisfies the system conformance requirement	Acceptable as long as it satisfies the system conformance requirement
pH	±0.2	±0.2
Wavelength	±3 nm	±3 nm
Salt Level	±10%	±10%
Mobile Phase	Choose the less one from ±30% or absolute quantity ±10%	Choose the larger one from ±30% or absolute quantity ±2%

■Nexera Method Scouting

Fig. 1 shows the scheme of the Nexera Method Scouting System. It is capable of automatically investigating up to 96 combinations of mobile phases and columns, without user intervention, thereby significantly improving method development productivity. In this application note, the reduction in analysis time for ibuprofen analysis was examined using a Shim-pack VP-ODS column compared to two core-shell analytical columns with different lengths, the Kinetex XB-C18 series.

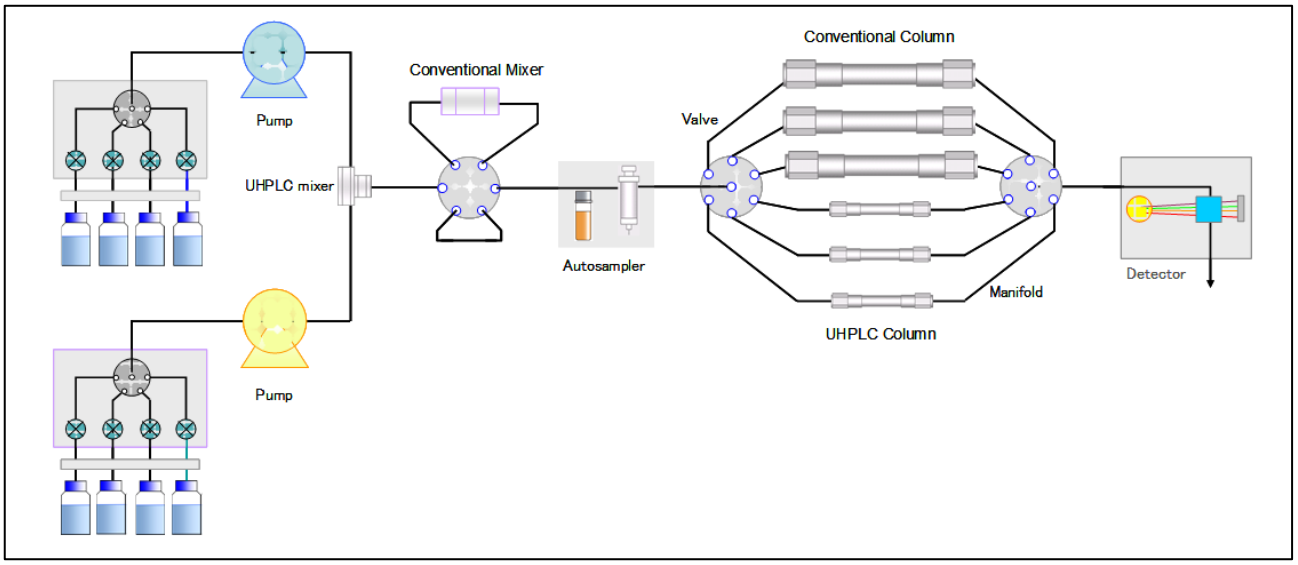


Fig. 1: Scheme of Nexera Method Scouting

■High Speed Analysis of Ibuprofen with Kinetex XB-C18

Ibuprofen is a non-steroidal anti-inflammatory drug (NSAID) used for fever reduction and pain relief. The ibuprofen analysis with the conventional column, L1 (C18), is found in the USP-NF[1)]. The method specifies that ibuprofen, its related compound C (4-Isobutylacetophenone), and valerophenone (internal standard) have to be analyzed at the same time. Fig. 2 shows the structural formulas of these components.

As shown in Table 1, USP <621> accepts the reduction of column particle size by up to 50 percent. Based on the ibuprofen analysis found in the USP-NF, the shortening of the analysis time was examined by only replacing the column with the core-shell column, Kinetex XB-C18 (particle size: 2.6 µm) without changing the flow rate (2.0 mL/min) or the composition of mobile phase. The internal diameter remained 4.6 mm.

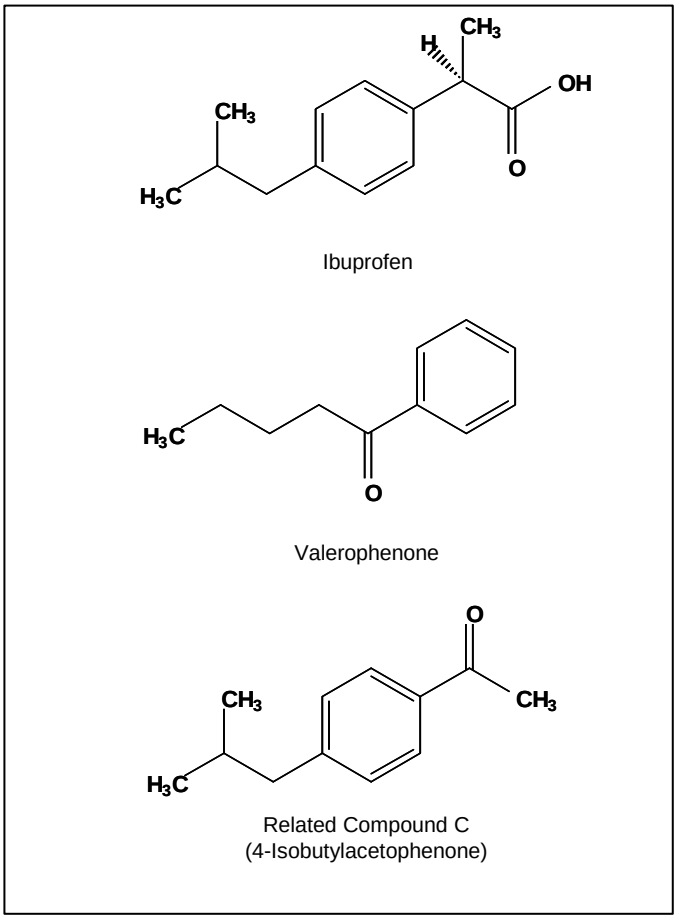


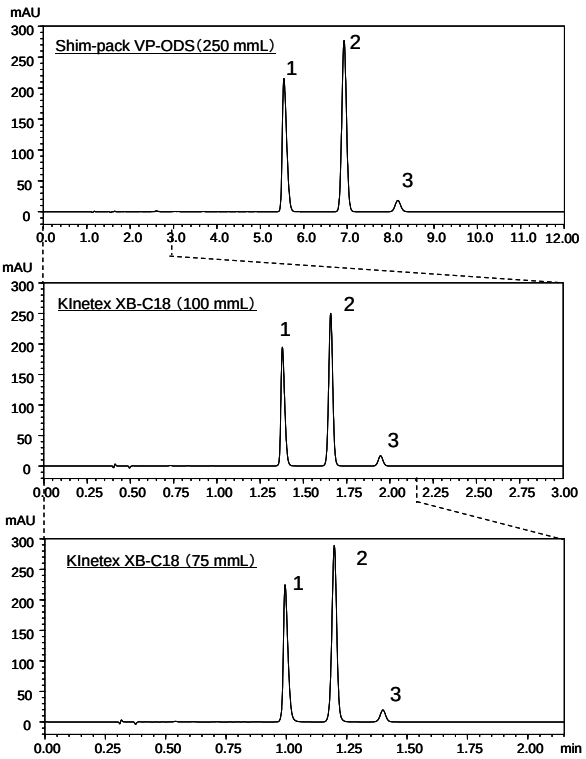
Fig. 2: Structures of Ibuprofen, valerophenone and related compound C (4-Isobutylacetophenone)

Fig. 3 shows the result of ibuprofen analysis using the conventional column, Shim-pack VP-ODS (particle size 4.6 µm, length 250 mm), and the core-shell columns, Kinetex XB-C18 (particle size 2.6 µm, length 100 mm) and Kinetex XB-C18 (particle size 2.6 µm, length 75 mm). Table 2 shows the analytical conditions.

The comparison of chromatograms between the upper (with Shim-pack VP-ODS) and the middle (Kinetex XB-C18, 100mm) shows that the analysis time per one cycle was shortened by approximately 75% without affecting the separation when using the core-shell column. Further, by using the core-shell column Kinetex XB-C18 (75 mm), which is 70 percent shorter than the conventional column (250 mm), the analysis time can be reduced by a factor of 6.

Table 2: Analytical Conditions

System	: Nexera Method Scouting
Column	: ①Shim-pack VP-ODS (250 mm L. × 4.6 mm i.d., 4.6 µm)
	: ②Kinetex XB-C18 (100 mm L. × 4.6 mm i.d., 2.6 µm)
	: ③Kinetex XB-C18 (75 mm L. × 4.6 mm i.d., 2.6 µm)
Mobile Phase	: A; 1%(wt / v) Chloroacetic Acid Water (pH 3.0 adjusted by ammonium hydroxide)
	B; Acetonitrile
	A / B = 2 / 3 (v / v)
Flow Rate	: 2.0 mL/min
Column Temp.	: 30℃
Injection Vol.	: ①5 µL
	②1 µL
	③1 µL
Detection	: SPD-20AV at 254 nm w/ high-sensitivity semi-micro cell



■Peaks
1. Ibuprofen (12 mg/mL)
2. Valerophenone (0.35 mg/mL)
3. Related Compound C (4-Isobutylacetophenone) (0.012 mg/mL)

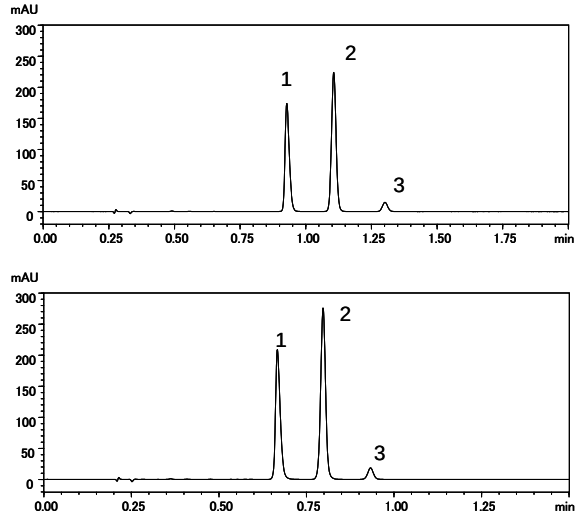
Fig. 3: Comparison of the Shim-pack VP-ODS and Kinetex XB-C18 series
- Chromatograms of a Standard Mixture of Ibuprofen, Valerophenone and Related compound C (4-Isobutylacetophenone) –

■Analysis of Ibuprofen with Increasing Flow Rate

The previous section showed the high-speed analysis of Ibuprofen by changing the column. In this example, a further reduction in analysis time was examined using the core-shell column, Kinetex XB-C18. Here the flow rate was changed to 3.0 mL/min, corresponding to the method-specified flow rate + 50 percent, based on the allowable range of USP <621> (cf. Table 1). Fig. 4 shows the resulting chromatogram and table 3 shows its conditions. When increasing the flow rate by 1.5 times, the analysis time per one cycle was reduced by over 30%.

Table 3: Analytical Conditions

System	: Nexera Method Scouting
Column	: Kinetex XB-C18 (100 mm L. × 4.6 mm i.d., 2.6 µm)
	: Kinetex XB-C18 (75 mm L. × 4.6 mm i.d., 2.6 µm)
Mobile Phase	: A; 1% (wt / v) Chloroacetic Acid Water (pH 3.0 adjusted by ammonium hydroxide)
	B; Acetonitrile
	A / B = 2 / 3 (v / v)
Flow Rate	: 3.0 mL/min
Column Temp.	: 30℃
Injection Vol.	: 1 mL
Detection	: SPD-20AV at 254 nm w/ high-sensitivity semi-micro cell



■Peaks
1. Ibuprofen (12 mg/mL)
2. Valerophenone (0.35 mg/mL)
3. Related Compound C (4-Isobutylacetophenone) (0.012 mg/mL)

Fig. 4: Chromatograms of a Standard Mixture of Ibuprofen, Valerophenone and Related compound C (4-Isobutylacetophenone)
Upper : Kinetex XB-C18 (100 mmL)
Lower : Kinetex XB-C18 (75 mmL)

The ibuprofen analysis found in the USP-NF describes certain evaluation criteria for system suitability: the resolution must be more than 2.5 between ibuprofen and valerophenone, and between valerophenone and the related compound C; also, the symmetry factors and the relative standard deviation (RSD) must be lower than 2.5 and 2.0, respectively, between each compound.

The high-speed analytical conditions using the Kinetex XB-C18 column satisfy all of the criteria for the system suitability standard of ibuprofen analysis found in the USP-NF. By using the core-shell column, it not only shortened the analysis time, but it also enabled reduction of solvent consumption by more than 75 percent.

Table 4 shows the summary of analysis.

Table 4: Summary of analysis

	System suitability	ShimPack VP-ODS	Kinetex XB-C18			
Column Length (mm)	-	250	75		100	
ID (mm)	-	4.6	4.6		4.6	
Particle Diameter (µm)	-	4.6	2.6		2.6	
Flow Rate (mL/min)	-	2.0	2.0	3.0	2.0	3.0
System back pressure (MPa)	-	18.5	24	37	30	45
Resolution (Peak 1 and Peak 2)	≧2.5	7.05	5.50	4.54	6.17	5.21
Resolution (Peak 2 and Peak 3)	≧2.5	5.60	4.96	4.36	5.82	5.18
Symmetry Factor (Ibuprofen)	≦2.5	1.44	1.36	1.34	1.37	1.28
Symmetry Factor (Valerophenone)	≦2.5	1.04	1.04	1.06	1.05	1.06
Symmetry Factor (Related Compound C)	≦2.5	1.04	1.02	1.04	1.04	1.06
RSD retention time (Ibuprofen)	≦2.0	0.038	0.059	0.088	0.045	0.067
RSD retention time (Valerophenone)	≦2.0	0.039	0.070	0.059	0.036	0.053
RSD retention time (Related Compound C)	≦2.0	0.045	0.066	0.056	0.030	0.045
RSD Area (Ibuprofen)	≦2.0	0.052	0.222	0.165	0.319	0.349
RSD Area (Valerophenone)	≦2.0	0.034	0.325	0.165	0.089	0.425
RSD Area (Related Compound C)	≦2.0	0.198	0.284	0.193	0.110	0.410
Analysis Time (min)	-	12.0	2.3	1.5	3.0	2.0
Solvent Consumption (mL)	-	24	4.6	4.5	6.0	6.0

■ Impurity Test of Ibuprofen Related Compound C

Any impurities present in pharmaceutical products are required to be tightly controlled due to the potential to adversely affect a product's qualities, such as stability, functionality, and availability.

In the impurity test of ibuprofen found in the USP-NF, the amount of the related compound C present is required to be less than 0.1%. Fig. 5 shows the analysis of an ibuprofen solution (12 mg/mL) using a UV-VIS absorbance detector (SPD-20AV) and the chromatogram enlarged from 0.5 minutes to 2 minutes. The impurity peak for compound C at 1.95 minutes was 0.039% and well below the limit.

Table 5 shows the analytical conditions. The SPD-20AV is a high-resolution and high-sensitivity detector with a wide dynamic range for simultaneous detection and quantitation of main components and impurities.

Table 5: Analytical Conditions

System	: Nexera Method Scouting
Column	: Kinetex XB-C18 (100 mm L. × 4.6 mm i.d., 2.6 µm)
Mobile Phase	: A; 1% (wt / v) Chloroacetic Acid Water (pH 3.0 adjusted by ammonium hydroxide) B; Acetonitrile A / B = 2 / 3 (v / v)
Flow Rate	: 2.0 mL/min
Column Temp.	: 30℃
Injection Vol.	: 10 µL
Detection	: SPD-20AV at 254 nm w/ high-sensitivity semi-micro cell

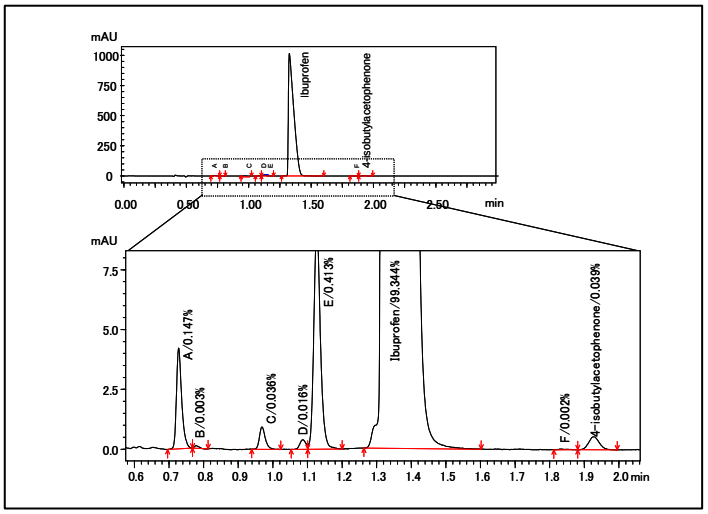


Fig. 5: Chromatograms of Ibuprofen

■ Repeatability

The SIL-30AC supports an injection volume range of 0.1 µL to 50 µL. Linearity is achieved throughout the injection range, from injection of a small volume for a UHPLC up to the order of several tens of µL used in conventional methods.

Table 6 shows the relative standard deviation (%RSD) for the peak area of the standard mixed solution of Ibuprofen (the concentration is equal to Fig. 5, 12 mg/mL) after 0.1µL injection repeated six times. The SIL-30AC has excellent repeatability even with an injection as small as 0.1µL.

Table 6: Repeatability of Peak Area（n=6）

	Ibuprofen	Valerophenone	Related Compound C (4-Isobutylacetophenone)
1st	26,090	35,914	35,914
2nd	25,936	35,308	35,308
3rd	25,829	35,263	35,263
4th	26,436	35,752	35,752
5th	26,087	35,387	35,387
6th	26,139	35,885	35,885
Average	26,086	35,585	35,585
%RSD	0.79	0.84	0.84

■ Reference

1) USP Monograph for Ibuprofen, USP35-NF30 2012

First Edition: December 2012

Shimadzu Corporation
www.shimadzu.com/an/

Related Products Some products may be updated to newer models.

Nexera series
Ultra High Performance Liquid Chromatograph

Related Solutions

- Price Inquiry
- Product Inquiry
- Technical Service / Support Inquiry
- Other Inquiry