SHIMADZU
Excellence in Science

Application News

No.L448

High Performance Liquid Chromatography

Ultra-High Speed Analysis of Ibuprofen by Nexera in Accordance with USP 621

High throughput analysis has been advancing dramatically in recent years with the increasing necessity to improve productivity and operational efficiency. HPLC has also been in the spotlight thanks to significant advances in ultra-high-speed analysis technology, in particular ultra high performance LC and micro-particle column packing material. The recently revised General Chapter 621 of the United States Pharmacopoeia (USP 621) now permits a degree of adjustment of HPLC and GC parameters, specifically aimed at satisfying the requirements of system suitability.
Here, using the Nexera ultra high performance liquid chromatograph and the Shim-pack VP-ODS conventional column, in addition to the Kinetex XB-C18 Series Core-Shell fast analysis column, we introduce examples of high-speed analysis of ibuprofen-related substances in conformance with USP 621.

Allowable Adjustments to HPLC Parameters

Table 1 shows the parameters which may be changed according to USP 621, such as column length, particle size and flowrate, etc., in addition to the actual permissible ranges within which these LC parameters may be changed. After the allowable changes are implemented, no re-validation is required since the changes are interpreted only as method adjustments.

Table 1 Allowable Adjustments to HPLC Parameters According to USP 621

	USP General Chapter <621>
Column Length	±70 % change allowed
Column Internal Diameter	Changes permitted as long as linear velocity is the same
Particle Size	May be reduced to 50 % at maximum. However, may not be increased.
Flowrate	±50 % change allowed
Column Temperature	±10 °C change allowed
Injection Volume	Change is permitted if it satisfies the requirements for system suitability.
pH	±0.2 change allowed
UV Wavelength	±3 nm change allowed
Salt Concentration	±10 % change allowed
Mobile Phase Composition	The smaller of ±30 % or ±10 % of absolute volume to be selected.

Table 2 Analytical Conditions

System	: Nexera Method Scouting
Column	: (1) Shim-pack VP-ODS (250 mmL. × 4.6 mm I.D., 4.6 µm) (2) Kinetex XB-C18 (100 mmL. × 4.6 mm I.D., 2.6 µm)
Mobile Phase	: A: 1 % (wt / v)Chloroacetic Acid Water (pH 3.0 adjusted with ammonium hydroxide) B: Acetonitrile A/B = 2/3 (v/v)
Flowrate	: 2.0 mL/min
Column Temp.	: 30 °C
Injection Vol.	: (1) 5 µL (2) 1 µL
Detection	: SPD-20AV at 254 nm

High Speed Analysis of Impurities in Ibuprofen with UHPLC Column

Ibuprofen is a type of non-steroidal anti-inflammatory drug (NSAID) that is used as an antipyretic and analgesic. Monographs on ibuprofen-related substances using conventional columns associated with the USP column category L1 (C18) are listed in the USP-NF[1)], in which the analysis method and system suitability are specified for three substances; ibuprofen and its degradation products 4-isobutyl acetophenone and valerophenone (internal standard substance).
This paper presents our investigation into speeding up the analysis of ibuprofen-related substances listed in USP-NF in compliance with USP 621. For the analytical column, we selected the Kinetex XB-C18 (100 mmL. × 4.6 mm ID, 2.6 µm) high-speed analytical column, which is within the acceptable range of column adjustment specified in USP 621. Except for the column, all other conditions were the same as those listed in USP-NF. Fig. 1 shows the results of analysis of a mixture of Ibuprofen, Valerophenone and 4-Isobutylacetophenone using the Shim-pack VP-ODS and Kinetex XB-C18 columns, respectively, and Table 2 shows the analytical conditions used for each. Use of the Kinetex XB-C18 made it possible to reduce both the analysis time and solvent consumption to about 1/4 without compromising the separation. The results of the system suitability test are shown in Table 3 on the following page. The results of this study clearly indicate that all of the system suitability requirements have been met using the Kinetex XB-C18.

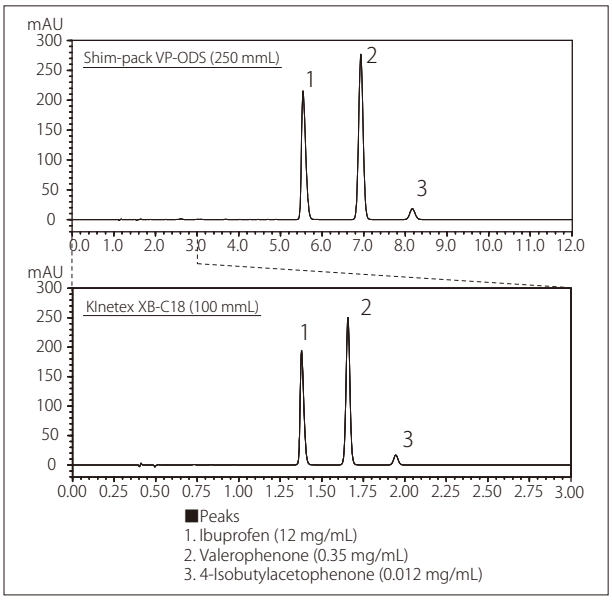

Fig. 1 Comparison of the Shim-pack VP-ODS and Kinetex XB-C18 Series Columns -Chromatograms of a Standard Mixture of Ibuprofen, 4-Isobutylacetophenone and Valerophenone
Upper: Shim-pack VP-ODS (250 mmL)
Lower: Kinetex XB-C18 (100 mmL)

Table 3 Permissible HPLC Adjustment Ranges According to USP 621

System Suitability Item			Reference Value	USP-Equivalent Condition	High-Speed Conditions
				ShimPack VP-ODS	Kinetex XB-C18
Relative Retention Time	Valerophenone (when the ibuprofen retention time is 1.0)		1.4	1.3	1.2
	4-Isobutylacetophenone (when the valerophenone retention time is 1.0)		1.2	1.2	1.2
Resolution	Ibuprofen and valerophenone		≧2.5	7.05	6.17
	Valerophenone and 4-isobutylacetophenone		≧2.5	5.60	5.82
Symmetry Factor	Ibuprofen		≦2.5	1.44	1.37
	Valerophenone		≦2.5	1.04	1.05
	4-Isobutylacetophenone		≦2.5	1.04	1.04
Relative Standard Deviation RSD (%)	Ibuprofen	Retention Time	≦2.0	0.028	0.039
		Peak Area Value	≦2.0	0.030	0.202
	Valerophenone	Retention Time	≦2.0	0.032	0.034
		Peak Area Value	≦2.0	0.026	0.087
	4-Isobutylacetophenone	Retention Time	≦2.0	0.036	0.028
		Peak Area Value	≦2.0	0.033	0.218

Ibuprofen Impurity Test

Impurities present in pharmaceuticals require strict management, as they can affect product quality in terms of stability, functionality and effectiveness. In the impurity test specified for ibuprofen in USP NF[1], the content of the ibuprofen decomposition product "4-isobutyl acetophenone" is required to be less than 0.1 % of the total.

Fig. 2 shows the results obtained from analysis of an Ibuprofen solution (12 mg/mL) using a UV-VIS absorbance detector (SPD-20AV), in which an expanded view of the chromatogram from approximately 0.5 minutes to 2 minutes is shown. The analytical conditions are shown in Table 4. With the wide dynamic range of the SPD-20AV, even the smallest peaks are clearly detected with high resolution and high sensitivity.

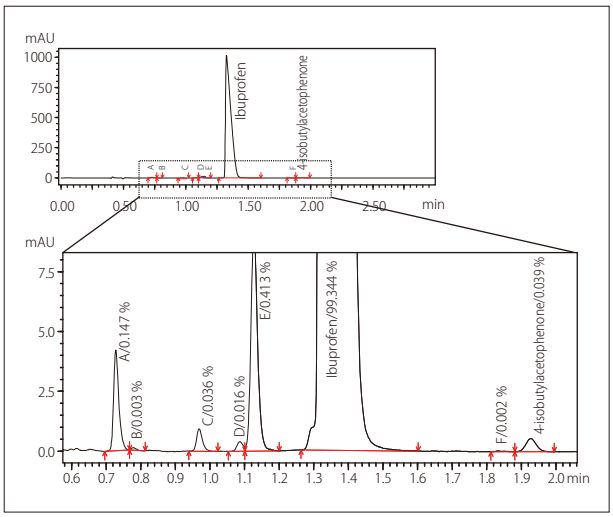

Fig. 2 Chromatograms of Ibuprofen and Impurities

Table 4 Analytical Conditions

System	: Nexera
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 with ammonium hydroxide) B: Acetonitrile A/B = 2/3 (v/v)
Flowrate	: 2.0 mL/min
Column Temp.	: 30 °C
Injection Vol.	: 10 µL
Detection	: SPD-20AV at 254 nm

[References]
1) U.S. Pharmacopeia 35-NF 30, 2012
· General Chapter <621>
· Official Monograph " Ibuprofen"

First Edition: May. 2013

SHIMADZU

Shimadzu Corporation
www.shimadzu.com/an/

For Research Use Only. Not for use in diagnostic procedures.
The content of this publication shall not be reproduced, altered or sold for any commercial purpose without the written approval of Shimadzu. The information contained herein is provided to you "as is" without warranty of any kind including without limitation warranties as to its accuracy or completeness. Shimadzu does not assume any responsibility or liability for any damage, whether direct or indirect, relating to the use of this publication. This publication is based upon the information available to Shimadzu on or before the date of publication, and subject to change without notice.

© Shimadzu Corporation, 2013