「Application Note」

Analysis of Ibuprofen by ChroZen HPLC & UHPLC According to USP 621

- HPLC & UHPLC Application

Abstract

Method validation in pharmaceutical industries takes a very critical role to ensure product quality and evaluate the system suitability for its intended purposes. There are several parameters included in analytical method validation such as specificity, accuracy, precision, system suitability, etc.

Most of all, system suitability is essentially required to verify whether the analytical methods configured with the instrument, operating parameters, and reference standards are performed under the guideline with appropriate acceptance. To confirm it, the reproducibility resulted from the replicate injection and the resolution between the analyte and internal standard are evaluated and some variation of analytical condition was applied within the allowable range to fit the requirements.

In this study, the analysis of Ibuprofen ablets was conducted by ChroZen HPLC and ChroZen UHPLC according to USP <621> to verify the system suitability for the application. Ibuprofen as a non-steroidal anti-inflammatory drug is widely used and its analysis is very routine in pharmaceuticals.

Instruments and Software

· ChroZen HPLC System

Item	Description	Part No.
Pump	ChroZen HPLC Quaternary Gradient Pump with Vacuum degasser	9421011020
Autosampler	ChroZen HPLC Autosampler	5421011020
Column oven	ChroZen HPLC Column oven for Analytical scale	3421011020
Detector	ChroZen HPLC UV/Vis Detector with dual wavelength	7411011020
Install. Option	HPLC Performance Kit (Without LC C18 Column)	1601011890
CDS	YL-Clarity software for single instrument of YCM HPLC	5301011000
	Autosampler control of YL-Clarity	5301011040
	System Suitability Test of YL-Clarity	5301011050
Column	C18 (4.6 mm x 250 mm, 5 μm)	-

· ChroZen UHPLC System

Item	Description	Part No.
Pump	ChroZen UHPLC Pump	9431011012
Autosampler	ChroZen UHPLC Cool/Heat Autosampler	9451011013
Column oven	ChroZen UHPLC Column oven for Analytical scale	3531011030
Detector	ChroZen UHPLC UV/Vis Detector	7521011020
Install. Option	UHPLC Performance Kit (Without LC C18 Column)	9361011150
CDS	YL-Clarity software for single instrument of YCM HPLC	5301011000
	Autosampler control of YL-Clarity	5301011040
	System Suitability Test of YL-Clarity	5301011050
Column	Poroshell C18 (2.1 mm x 100 mm, 2.7 μm)	-

[ChroZen HPLC]

[ChroZen UHPLC]

Reagents and Standards

- Acetonitrile, HPLC Grade
- Ammonium hydroxide solution, 28.0-30.0%
- Chloroacetic acid, 99.0%
- Ibuprofen, Pharmaceutical secondary STD
- Valerophenone, 99.0%
- Ultrapure water, 18.2 MΩ-cm resistivity

Preparation

1. Mobile phase

① Dissolve 4.0 g of Chloroacetic acid in 400 mL of water.

② Adjust a pH to 3.0 with Ammonium hydroxide.

③ Add 600 mL of Acetonitril to ② solution and mix well. Then, filtrate and degas the mixture prior to use.

2. Internal standard solution

Dissolve Valerophenone in the mobile phase to make the concentration to 0.35 mg/mL.

3. Standard preparation

Dissolve the Ibuprofen standard in an internal standard solution to have a concentration of 12 mg/mL.

4. Assay preparation

① Grind more than 20 tablets of Ibuprofen and accurately weigh 1200 mg.

② Add 100 mL of Internal standard solution and mix for 10 minutes.

③ Centrifuge the solution and separate the top layer for use.

Instrument Conditions & Chromatogram

ChroZen HPLC System	
Mobile phase	10% Chloroacetic acid(pH 3, NH_4OH) : ACN = 40 : 60
Flow rate	2.0 mL/min
Column	C18 (4.6 mm x 250 mm, 5 µm)
Temperature	30°C
Injection volume	5 µL
Detection	UV/Vis detector 254 nm

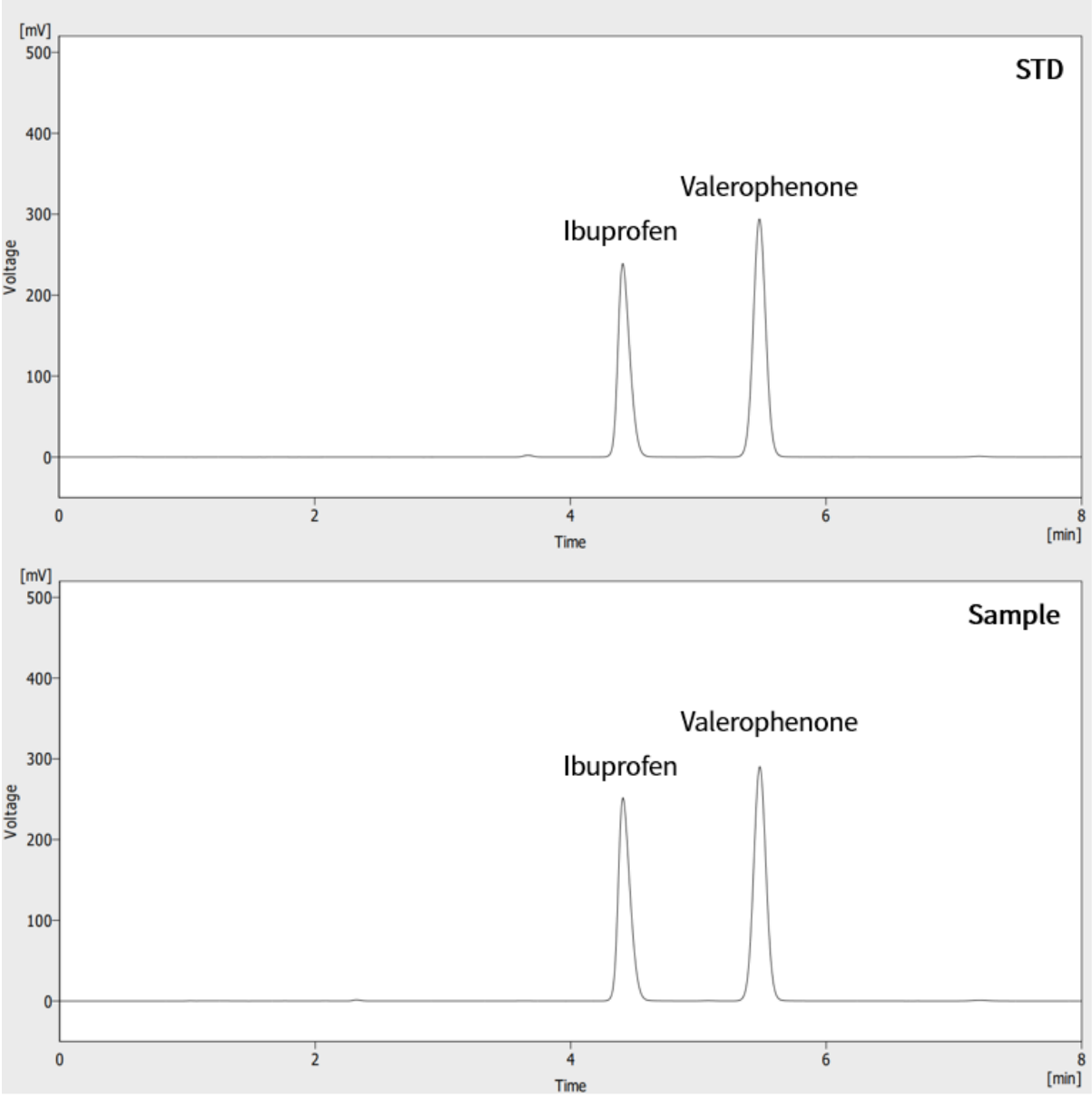

Figure 1. Standard & Sample Chromatograms of Ibuprofen and valerophenone(ISTD)by ChroZen HPLC

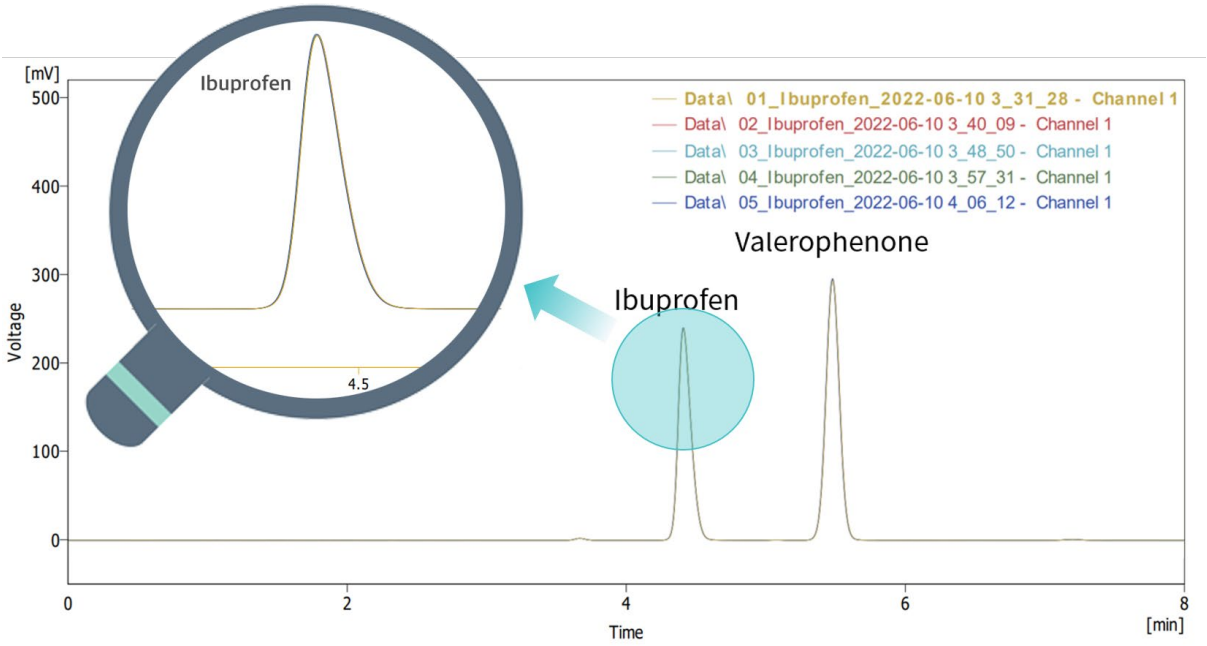

Figure 2. Overlay of 5 replicate injections of the standard chromatograms by ChroZen HPLC

Table 1. Validity of Test Method for 5 replicate injections by ChroZen HPLC

5 replicate injections	Resolution	Area RSD (%)	
		Ibuprofen	Valerophenone
USP Regulation	≥ 2.5	≤ 2.0	≤ 2.0
Result	6.04	0.293	0.267
	Pass	Pass	Pass

Table 2. Validity of Test Method for 20 replicate injections by ChroZen HPLC

20 replicate injections	Resolution	Area RSD (%)	
		Ibuprofen	Valerophenone
Result	6.04	0.323	0.319

ChroZen UHPLC System	
Mobile phase	10% Chloroacetic acid(pH 3, NH_4OH) : ACN = 40 : 60
Flow rate	0.6 mL/min
Column	Proshell C18 (2.1 mm x 100 mm, 2.7 µm)
Temperature	30°C
Injection volume	1 µL
Detection	UV/Vis detector 254 nm

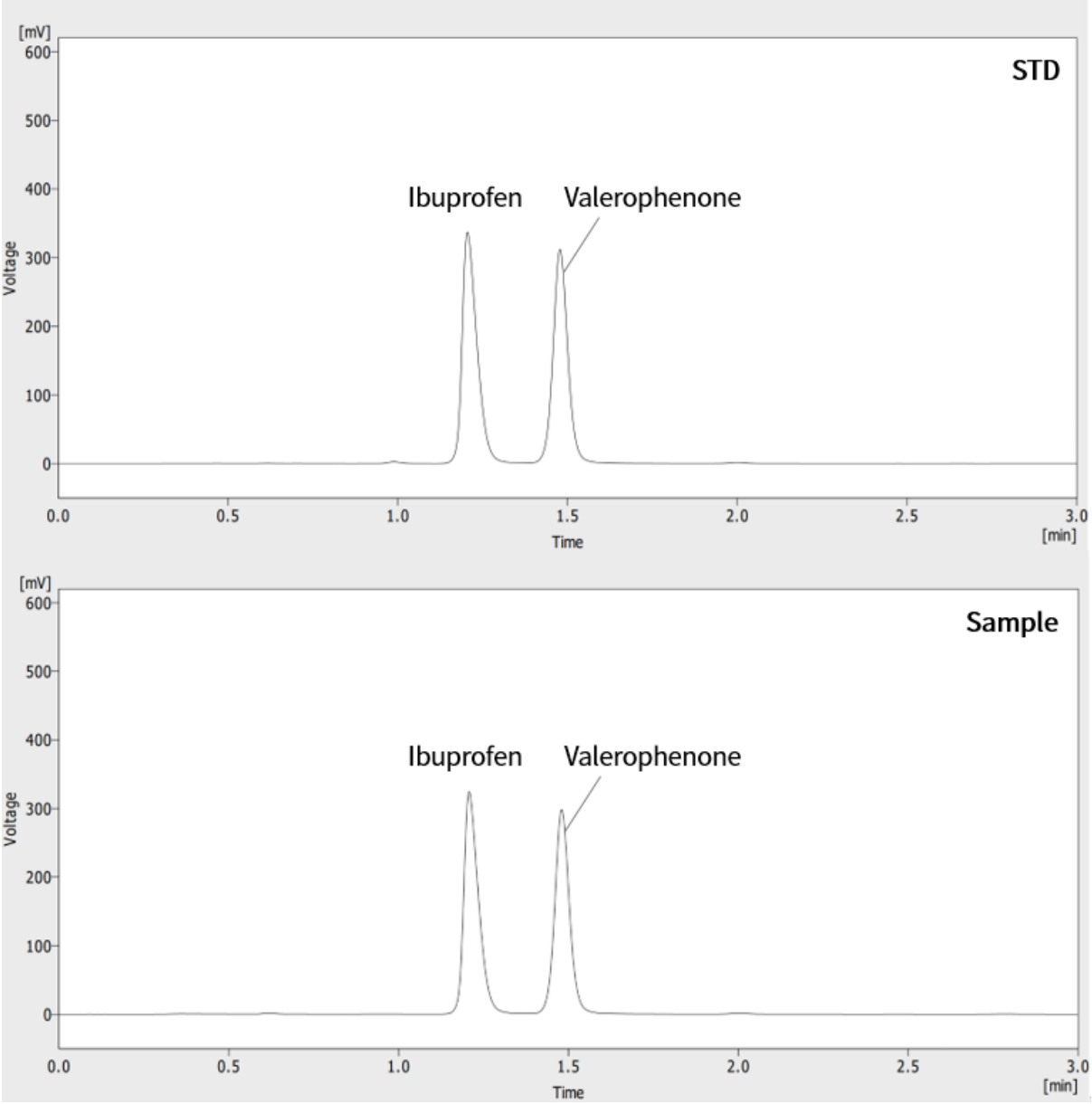

Figure 3. Standard & Sample chromatograms of Ibuprofen and valerophenone(ISTD) by ChroZen UHPLC

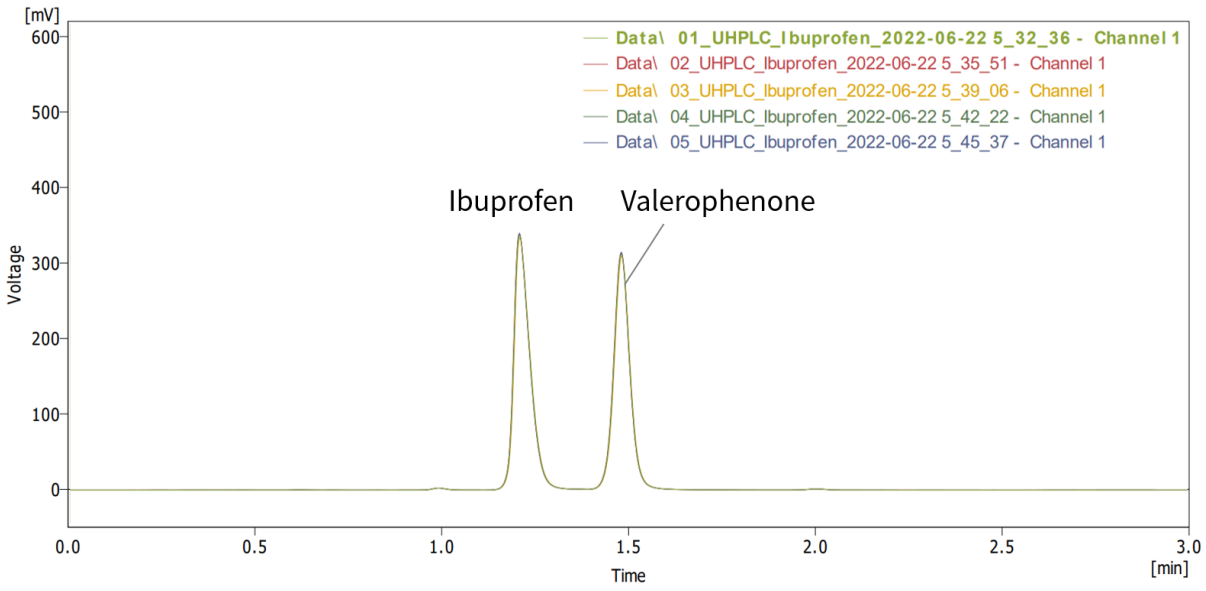

Figure 4. Overlay of 5 replicate injections of the standard chromatograms by ChroZen UHPLC

Table 3. Validity of Test Method for 5 replicate injections by ChroZen UHPLC

5 replicate injections	Resolution	Area RSD (%)	
		Ibuprofen	Valerophenone
USP Regulation	≥ 2.5	≤ 2.0	≤ 2.0
Result	3.42	0.638	0.582
	Pass	Pass	Pass

System Suitability

	USP <621>	HPLC Condition	UHPLC Condition
pH of mobile phase	± 0.2	As specified	As specified
Concentration of salts in buffer	± 10%	As specified	As specified
Ratio of components in mobile phase	± 30% Relative Cannot exceed ± 10%	As specified	As specified
Wavelength of UV-visible detector	± 3 nm	As specified	As specified
Column length	*Isocratic separation* ; Column length(L) to particle size diameter(dp) ratio can be adjusted between -25% and 50% *Gradient separation* ; Not allowed	As specified	As specified
Column inner diameter	Can be adjusted if the linear velocity maintained	As specified	2.1 mm (As specified)
Particle size	*Isocratic separation* ; Column length(L) to particle size diameter(dp) ratio can be adjusted between -25% and 50% *Gradient separation* ; Not allowed	As specified	2.7 µm (As specified)
Flow rate	± 50%(Isocratic only)	As specified	0.6 mL/min (As specified)
Injection volume	Can be adjusted as far as it is consistent with aceepted precision, linearity, and detection limit	As specified	1 µL (As specified)
Column temperature	± 10°C	As specified	As specified

Conclusion

This study is performed to verify the system suitability for the analysis of Ibuprofen by ChroZen HPLC and ChroZen UHPLC.

The analysis method for ChroZen HPLC was applied as specified in USP 621. Resolution between ibuprofen and valerophenone resulted in 6.04 and area reproducibility (% RSD) of ibuprofen and valerophenone was 0.293 and 0.267 which reach satisfactory results for USP requirements [Table 1]. To confirm the system reliability, 20 replicate injections were conducted and the result was similar to one of 5 replicate injections [Table 2].

Analysis of Ibuprofen was also performed by ChroZen UHPLC as specified in USP 621. The condition for the column and mobile phase was adjusted within the allowable variation range of the analysis condition. Resolution between ibuprofen and valerophenone resulted in 6.04 and area reproducibility (% RSD) of ibuprofen and valerophenone was 0.638 and 0.582. This was also satisfied with the USP requirements [Table 2]. With ChroZen UHPLC, the consumption of mobile phase and solution was significantly reduced, and so was the analysis time, which improves productivity.

As a result, both ChroZen HPLC and ChroZen UHPLC are fully suitable for the analysis of Ibuprofen and the system needs to be selected depending on users' preferences and conditions.

Reference

- USP <621> CHROMATOGRAPHY

- USP Ibuprofen, Ibuprofen tablets

- Korean Pharmaceuticals

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