Analysis of USP Levocetirizine Dihydrochloride Tablets – ORGANIC IMPURITIES Modifying the Method for Fast Analysis in Accordance with USP General Chapter <621>

The United States Pharmacopoeia (USP) and the European Pharmacopoeia (EP) permit a degree of adjustment of HPLC parameters such as column size, mobile phase condition etc. if it is satisfying the requirements of system suitability. Allowable adjustment parameters are described in General Chapter <621> Chromatography of USP 41 and General Chapter 2.2.46 Chromatographic separation techniques of EP 9 each. Although both Pharmacopoeias permit to modify parameters, the allowable adjustments range may be different. For example, it is possible to modify the column size under the gradient condition in EP, but not allowed in USP. Modifying these analytical parameters within range is expected to reduce the analysis time.
This Technical Note introduces an example of analysis of Levocetirizine Dihydrochloride Tablets -ORGANIC IMPURITIES- in accordance with the USP. And an example of analysis that can be completed in a shorter analysis time after modifying the condition which is based on the USP General Chapter <621> Chromatography.

(K. Kanno)

Allowable adjustments range in USP

According to the USP General Chapter <621> Chromatography, it is possible to modify the specific parameters as below tables if it is satisfying the requirements of system suitability.

-Allowable adjustments range of HPLC column parameters

	Isocratic	Gradient
Stationary Phase	Not allowed	Not allowed
Particle size (dp, mm)	**L/dp ratio constant or Theoretical plate number : -25 to + 50%**	
Length (L, mm)		
Inner diameter (dc, mm)	**Can be adjusted if the linear velocity is kept constant.**	

-Allowable adjustments range of HPLC system parameters

	Isocratic	Gradient
Flow rate (F, mL/min)	**Flow rate changes for both a change in column diameter and particle size can be made by:** $F_2 = F_1 \times [(dc_2^2 \times dp_1)/(dc_1^2 \times dp_2)]$ **Additionally, F_2 can be adjusted by ±50%**	Not allowed
Injection Vol.	**Can be adjusted as far as it is consistent with accepted precision, linearity, and detection limits.**	
Temperature	**±10 ℃**	
Wavelength of UV	Not allowed	

F_1 Original Flow rate, µL/min
F_2 Modified Flow rate, µL/min
dp_1 Original Particle size, mm
dp_2 Modified Particle size, mm
dc_1 Original Inner Diameter, mm
dc_2 Modified Inner Diameter, mm

-Allowable adjustments range of mobile phase parameters

	Isocratic/Gradient
pH	**±0.2**
Concentration of Salts in Buffer	**Within ±10 % if the permitted pH variation**
Ratio of Components	**The amounts of minor components (specified at 50 % or less) can be adjusted by ±30 % relative. However, the change in any component cannot exceed ±10 % absolute.**

The above tables are based on the USP41 NF36 General Chapter <621> Chromatography.

Example: USP Levocetirizine Dihydrochloride Tablets – ORGANIC IMPURITIES

This is an example of analysis of Levocetirizine Dihydrochloride Tablets -ORGANIC IMPURITIES- in accordance with the USP General Chapter <621> Chromatography. The USP specified condition were modified in the following procedure, and the modified condition for fast analysis were completed.
USP specifies a 150 mm Length (L) × 4.6 mm Inner diameter (dp); 5 µm Particle size (dc) column operated at 1.0 mL/min. So L/dp is 50,000 and this value remains into the range between −25 % to +50 % as below.

$$37{,}500 \leqq L/dp \leqq 75{,}000$$

The table below is an example of column size within the above L/dp range.

Length (L, mm)	I.D. (dc, mm)	Particle size (dp, µm)	Relative Values	
			L/dP	Flow rate (F, mL/min)
250	4.6	5	50,000	1.0
150	4.6	3	50,000	1.7
150	3.0	3	50,000	0.7
150	2.1	3	50,000	0.3
125	3.0	3	41,700	0.7
100	4.6	2	50,000	2.5
100	3.0	2	50,000	1.1
100	2.1	2	50,000	0.5
75	3.0	2	37,500	1.1

As shown in the above table, you can select from multiple column sizes for modifying the method. The column is selected the most suitable one depending on the column line up and HPLC instrument condition such as maximum pressure. This time, the column shown below table was selected for fast analysis.

	USP Specified condition	Allowable adjustments range	Modified condition
Length (L, mm)	250 mm	In case of 3 µm L: 113-225 mm	150 mm
Particle size(dp, µm)	5 µm		3 µm
L/dp	50,000		50,000
I.D. (dc, mm) Flow rate (F, mL/min)	4.6 mm 1.0 mL/min	In case of 3.0 mm I.D. F: 0.35-1.06 mL/min	3.0 mm 1.0 mL/min

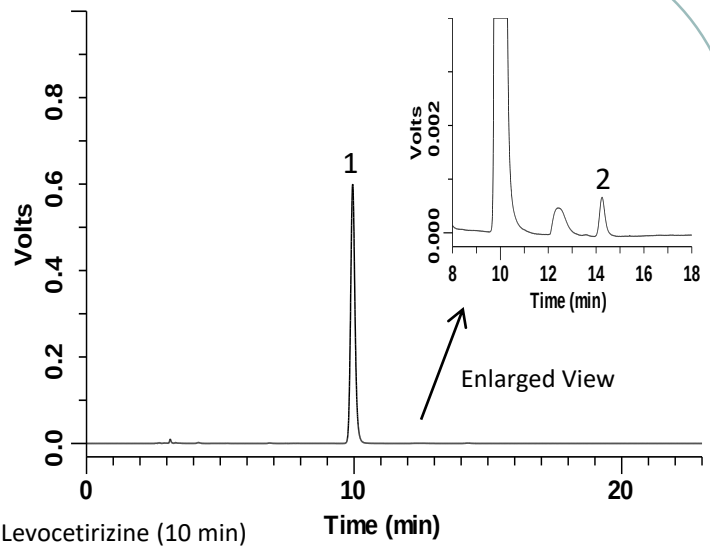
-Chromatograms-

The retention time of USP specified condition is about 23 minutes. On the other hand, the retention time of modified condition is about 5.75 minutes and the analysis time was reduced about 75 %.
The analysis time of this application will be longer because the USP levocetirizine tablets organic impurity test specifies that analysis is performed 2.3 times the retention time of levocetirizine.
Furthermore, this analysis takes a long time to equilibrate the column because it uses unmodified silica-gel as HILIC mode. It is recommended to flow the mobile phase more than 30 times of empty column volume for column equilibration.
Therefore, the time of analysis and equilibration are reduced by modifying the condition and using smaller size column.

1. Levocetirizine (0.2 mg/mL)
2. Chlorobenzhydryl Piperazine (0.2 µg/mL)

USP Specified HPLC Condition

Column : Inertsil SIL-100A
(5 µm, 250 x 4.6 mm I.D.)
Cat. No. : 5020-01712
Mobile phase : CH_3CN/H_2O/1M H_2SO_4 in H_2O
=93/6.6/0.4, v/v/v
Flow rate : 1.0 mL/min
Temperature : 30 ℃
Detection : UV 230 nm
Injection Vol. : 20 mL

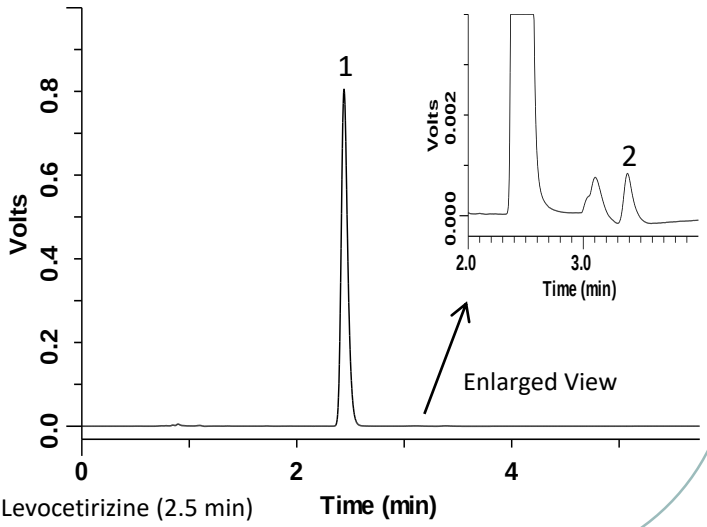


Analysis time is 2.3 times the retention time of Levocetirizine (10 min)
→ **23 min**

Fast analysis and High-Throughput

Modified HPLC Condition

Column : Inertsil SIL-100A
(3 µm, 150 x 3.0 mm I.D.)
Cat. No. : 5020-04225
Mobile phase : CH_3CN/H_2O/1M H_2SO_4 in H_2O
=93/6.6/0.4, v/v/v
Flow rate : **1.0 mL/min**
Temperature : 30 ℃
Detection : UV 230 nm
Injection Vol. : **10 mL**



Analysis time is 2.3 times the retention time of Levocetirizine (2.5 min)
→ **5.75 min**

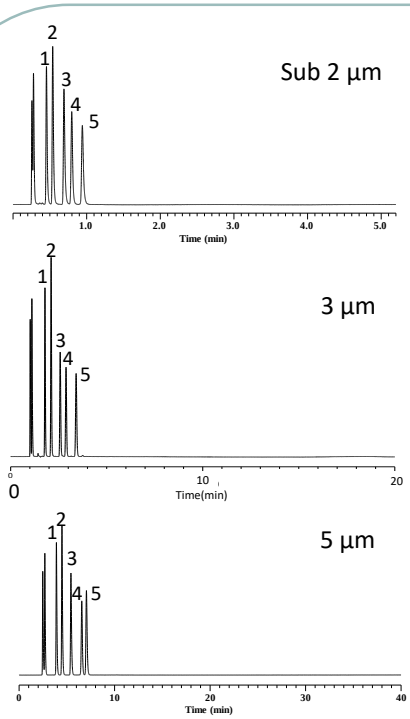
- Result of System Suitability Test

The results of the USP Specified method and the Modified method for fast analysis are shown in the table as below. These results are clearly satisfied that all of the system suitability requirements.

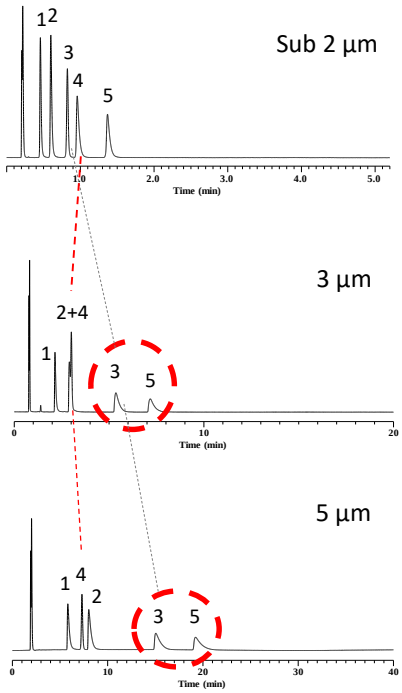
		Criteria	USP Specified method		Modified method for Fast analysis	
Resolution between peak1 and peak2		Not less than 3	11.7	PASS	7.31	PASS
Tailing factor of peak 1		Not more than 2	1.21	PASS	1.28	PASS
RSD%	Peak 1	Less than 1.0 %	0.18 %	PASS	0.05 %	PASS
	Peak 2	Less than 5.0 %	2.11 %	PASS	0.89 %	PASS

In generally, we select a smaller particle size column when modifying the method for fast analysis. Therefore, it is the most important to modify condition using the columns that have same separation pattern between different particle sizes.

Even with the use of same name column, separation patterns may change between different particle sizes. One of the reasons may be that the quality of bare silica-gels are difference with between particle sizes.
GL Sciences has been synthesizing base silica-gels . So we can recommend the LC column of Inertsil / InertSustain series for modifying method analysis between the different particle sizes such as USP.



InertSustainSwift C18



Example of Commercial C18 column

Condition

Eluent : A) CH_3CN
B) 25 mM K_2HPO_4
(pH 7.0, KH_2PO_4)
A/B = 60/40,v/v
Flow Rate : *1
Col. Temp. : 40 ℃
Detection : UV 220 nm
Injection Vol : *2

1. Chlorpheniramine
2. Triprolidine
3. Homochlorcyclizine
4. Hydroxyzine
5. Clemastine

Sub 2 µm (2.1 × 50 mm)
*1 : 0.4 mL/min ※2 : 0.5 µL
3 µm (2.1 × 150 mm)
*1 : 0.3 mL/min ※2 : 1 µL
5 µm (4.6 × 250 mm)
*1 : 1.0 mL/min ※2 : 2 µL

HPLC Column

Cat.No. 5020－01712	Inertsil SIL-100A	5 µm, 250 × 4.6 mm I.D.
Cat.No. 5020－04225	Inertsil SIL-100A	3 µm, 150 × 3.0 mm I.D.

Shipping solvent of Inertsil SIL-100A is n-Hexane/Ethanol. At first, sufficient flashing with Ethanol is required before flowing with the mobile phase of Levocetirizine analysis due to the mobile phase is Acetonitrile/Water. It is recommended to flow the mobile phase more than 30 times of empty column volume for column equilibration.

-Empty column volume-
250 × 4.6 mm I.D. : Approx. 4.2 mL
150 × 3.0 mm I.D. : Approx. 1.1 mL

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