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Application News

High-performance liquid chromatograph i-Series LC-2050C

Analysis of Valacyclovir Hydrochloride in Accordance with Japanese Pharmacopoeia

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User Benefits

- Analysis of valacyclovir hydrochloride according to the Japanese Pharmacopoeia can be performed easily and sensitively.
- Reliable repeatability can be obtained even in column oven conditions below room temperature.

■ Introduction

Valaciclovir hydrochloride is used to treat herpes simplex, varicella and shingles. This drug is a DNA polymerase inhibitor that suppresses the growth of viruses by inhibiting DNA replication, aiming to reduce the growth of herpes viruses that cause herpes simplex, varicella and shingles.

In this article, we present an analysis using an integrated liquid chromatograph "i-Series LC-2050C" and an option kit for low temperature analysis in accordance with the Japanese Pharmacopoeia 18th edition. In this test, all the analyses shall be carried out below room temperature.

■ Analysis in Accordance with Japanese Pharmacopeia

The Japanese Pharmacopoeia specifies "purity tests" and "quantitative tests" using a liquid chromatograph. These tests are required to confirm system suitability such as "confirmation of detection," "system performance," and "system repeatability.". System suitability tests for valacyclovir hydrochloride analysis are as follows:

Purity (ii):

In the 'Confirmation of detection' section, analyze both the solution[*1] for purity test (ii) (4.0 mg/L, prepared from 1 mL[*2] of sample solution with the preparation mixture[*3]) and its 20-fold diluted solution with the preparation mixture to confirm the peak area ratio of valacyclovir.

In 'System performance', the solution for purity test (ii) is analyzed to confirm the theoretical plate number and symmetry factor of the valacyclovir peak.

In 'System reproducibility', the solution for purity test (ii) is analyzed six times repeatedly to confirm the relative standard deviation of the peak areas.

Purity (iii):

In the 'Confirmation of detection' section, both the sample solution[*2] and the solution obtained by diluting the solution for purity test (iii)[*4] (4 mg/L, prepared from 1 mL[*2] of the sample solution with 0.05 mol/L HCl solution) with 0.05 mol/L HCl solution are analyzed to confirm the peak area ratio of valacyclovir.

In 'System performance', the solution for purity test (iii) is analyzed to confirm the theoretical plate number and symmetry factor of the valacyclovir peak.

In 'System reproducibility', the solution for purity test (iii) is analyzed six times repeatedly to confirm the relative standard deviation of the peak area values.

Quantitative method:

In System Performance, measure the standard solution for assay[*5] (500 mg/L, prepared with 0.05 mol/L hydrochloric acid TS) and check the number of theoretical plates and symmetry factor of the peak of valacyclovir.

In System Reproducibility, the standard solution[*5] is analyzed six times repeatedly to determine the relative standard deviation of peak area value.

＊1 Solution for Purity (ii):
To 1 mL of the sample solution add the mixture for preparation to make 100 mL.

＊2 Sample solution:
Dissolve 40 mg of valacyclovir hydrochloride in 100 mL of the preparation mixture.

＊3 Mixture for preparation:
A mixture of water and ethanol (95) (4: 1).

＊4 Solution for Purity (iii):
Measure exactly 1 mL of the sample solution and add 0.05 mol/L hydrochloric acid TS to make exactly 100 mL.

＊5 Standard solution for assay:
Weigh accurately about 25 mg of Valacyclovir hydrochloride Reference Standard, and dissolve in 0.05 mol/L hydrochloric acid TS to make exactly 50 mL.

Table 1 shows the analytical conditions for the purity test (ii), and Table 2 shows the analytical conditions for the purity test (iii) and the assay. In this paper, we employed the analytical conditions in Tables 1 and 2.

Table 1 Analytical Conditions for Purity (ii)

System	: i-Series LC-2050C
Column	: Shim-pack™ VP-Phenyl ＊6 (250 mm x 4.6 mm I.D., 5 µm)
Mobile Phase A	: Trifluoroacetic acid / water = 3 g / 1000 mL
Mobile Phase B	: Trifluoroacetic acid / methanol = 3 g / 1000 mL
Time Program	: B Conc. 10% (0 – 5 min) → 40% (5 – 35 min)
Flow Rate	: 0.8 mL/min
Column Temp.	: 15°C
Injection Volume	: 10 µL
Detection	: UV at 254 nm

＊6 P/N：228-59928-92

Table 2 Purity (iii) and assay conditions

System	: i-Series LC-2050C
Column	: CROWNPAK CR(+) (150 mm x 4.0 mm I.D., 5 µm)
Mobile Phase	: Perchloric acid / methanol / water = 5 mL/30 mL /1000 mL
Flow Rate	: 0.7 mL/min ＊7
Column Temp.	: 10°C
Injection Volume	: 10 µL
Detection	: UV at 254 nm

＊7 Adjusted to make valaciclovir retention 21 min.

■ Analytical Results

Table 3 System suitability test results

Test		Test item	Criteria	Result	Judgement
Purity (ii)	Detectability	Area ratio	3.5% - 6.5%	4.92%	PASSED
	System performance	Theoretical plate number	≥ 25000	46742	PASSED
		Symmetry factor	≤ 2.0	1.02	PASSED
	System repeatability	Relative standard deviation	≤ 2.0%	0.49%	PASSED
Purity (iii)	Detectability	Area ratio	0.07% - 0.13%	0.093%	PASSED
	System performance	Theoretical plate number	≥ 700	1941	PASSED
		Symmetry factor	≤ 1.5	1.08	PASSED
	System repeatability	Relative standard deviation	≤ 2.0%	0.64%	PASSED
Assay	System performance	Theoretical plate number	≥ 700	1849	PASSED
		Symmetry factor	≤ 1.5	1.22	PASSED
	System repeatability	Relative standard deviation	≤ 1.0%	0.02%	PASSED

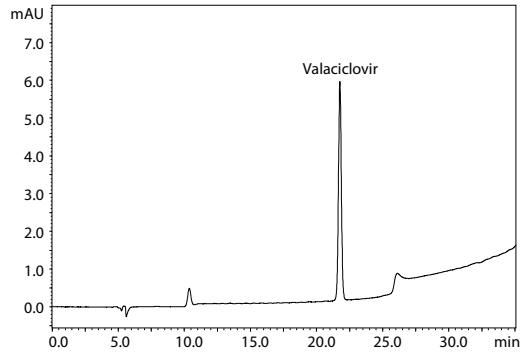

Figure 1 Chromatogram in accordance with the Japanese Pharmacopoeia Purity (ii) - System performance

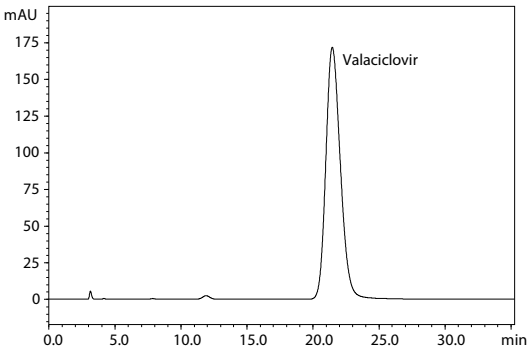

Figure 2 Chromatogram in accordance with the Japanese Pharmacopoeia Purity (iii) - System performance

Table 3 shows the results of the system suitability test, Figure 1 shows the chromatogram of the purity test (ii) -system performance, Figure 2 shows the purity test (iii) -chromatogram of system performance, and Figure 3 shows chromatogram of system performance –assay.

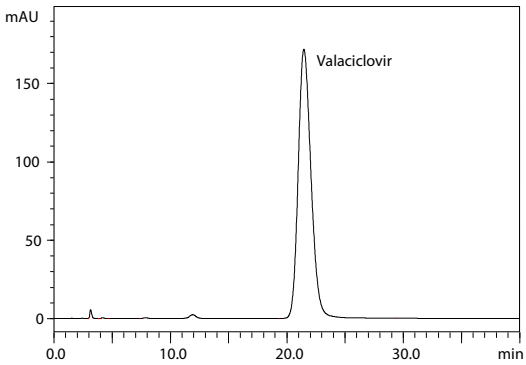

Figure 3 Chromatogram in accordance with the Japanese Pharmacopoeia Quantitative methods - system performance

■ Conclusion

In this paper, analysis of valacyclovir hydrochloride was carried out using the i-SeriesLC-2050C integrated liquid chromatograph, which was in accordance with the Japanese Pharmacopoeia's system compatibility test for valacyclovir hydrochloride. The results confirmed that the detection, system performance and system reproducibility all met the criteria of the Japanese Pharmacopoeia.

In order to set the column oven temperature below 15°C, the i-series low-temperature analysis option kit was used. This allows the column oven to be set to 10°C for analysis in typical laboratory environment (25°C).

[Precautions]

The column used in this paper, CROWNPAK CR (+), is a column packed with silica gel immobilized with 18 crown ether for liquid chromatography. For more information, please contact the manufacturer, Daicel Corporation.

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