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Application News

High Performance Liquid Chromatography

Analysis of Cortisone Acetate Tablets by Nexera UC SFC System

No. HPLC-010

The United States Pharmacopeia (USP) monographs are widely referenced to ensure the quality of drug substances. The USP monographs offer reliable and robust methods, but some methods, especially those using normal phase HPLC, require harmful solvents. Many laboratories have desired to reduce the use of such solvents for health, safety, and cost.
This application note shows an example of the analysis of cortisone acetate tablets according to the USP monograph. The original normal phase method was transferred to an SFC method with a conventional column, Shim-pack CLC-SIL, then a high-speed column, Shim-pack XR-SIL by using the Nexera UC SFC system.

■Method transfer from Normal Phase Mode to SFC

The USP monograph for cortisone acetate tablet analysis specifies a 4.6 mm x 25 cm silica column (L3) and n-butyl chloride/water-saturated n-butyl chloride/tetrahydrofuran /methanol/glacial acetic acid=95/95/14/7/6 mobile phase. This normal phase method was transferred to SFC method parameters. Tables 1 and 2 show the analytical conditions for USP method and SFC method, respectively. The same column (Shim-pack CLC-SIL) was used for both of the methods. Fig. 1 shows the comparison of chromatograms of system suitability solution (methylparaben 0.01 mg/mL, cortisone acetate 0.12 mg/mL, hydrocortisone acetate 0.1 mg/mL) between the USP method and the SFC method. The analysis time was shortened from 8.5 min to 3.6 min. Table 3 shows the system suitability result. The SFC method satisfies all of the criteria for the system suitability requirement in the USP-NF. By transferring to SFC and a CO_2/Methanol mobile phase, complex mixtures of harmful organic solvents with costly waste disposal can be changed to clean and inexpensive solvents.

Table 1: Analytical Conditions (Normal Phase Method)

System	: Prominence
Column	: Shim-pack CLC-SIL (250 mm L.×4.6 mm I.D., 5 µm)
Mobile Phase	: n-Butyl chloride/water-saturated n-butyl chloride /tetrahydrofuran/methanol/glacial acetic acid =95/95/14/7/6
Flow Rate	: 1.0 mL/min
Column Temp.	: Room temperature
Injection Vol.	: 15 µL
Detection	: UV 254 nm
Flow Cell	: High-pressure cell for SFC

Table 2: Analytical Conditions (SFC Method 1)

System	: Nexera UC
Column	: Shim-pack CLC-SIL (250 mm L.×4.6 mm I.D., 5 µm)
Mobile Phase	: A. CO_2, B. Methanol, A/B=85/15
Flow Rate	: 3.0 mL/min
Column Temp.	: 40 °C
Injection Vol.	: 2 µL
Detection	: UV 254 nm
Flow Cell	: High-pressure cell for SFC

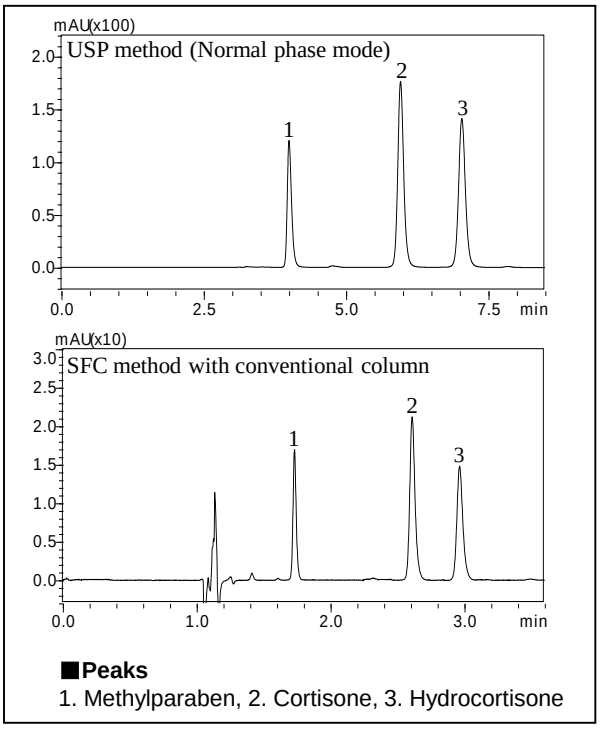

Fig.1: Chromatogram of system suitability solution (Upper: USP method, lower: SFC method with conventional column)

Table 3: System suitability results

System suitability requirements		CLC-SIL Prominence	CLC-SIL Nexera UC
USP resolution between cortisone acetate and hydrocortisone acetate	≥ 2.2	5.33	4.64
Relative standard deviation for cortisone acetate	≤ 2.0 %	Rt 0.062 %	Rt 0.041 %
		Area 0.133 %	Area 0.327 %
Relative standard deviation for hydrocortisone acetate	≤ 2.0 %	Rt 0.078 %	Rt 0.052 %
		Area 0.142 %	Area 0.448 %

■ Additional Run Time Reduction

A high speed column with small particles was used on the Nexera UC system to further shorten the run time. Table. 4 shows the analytical conditions. Fig. 2 shows the comparison of chromatograms between SFC method with conventional column (Shim-pack CLC-SIL) and high-speed column (Shim-pack XR-SIL). The run time went from 3.6 min to 0.62 min – almost 6 times shorter while maintaining resolution. Table 5 shows the system suitability result. The SFC method with a high-speed column satisfies all of the criteria for the system suitability requirement in the USP-NF.

Table 4: Analytical Conditions (SFC Method 2)

System	: Nexera UC
Column	: Shim-pack XR-SIL (100 mm L.×3.0 mm I.D., 2.2 μm)
Mobile Phase	: A. CO_2, B. Methanol, A/B=85/15
Flow Rate	: 3.0 mL/min
Column Temp.	: 40 °C
Injection Vol.	: 1 μL
Detection	: UV 254 nm
Flow Cell	: High-pressure cell for SFC

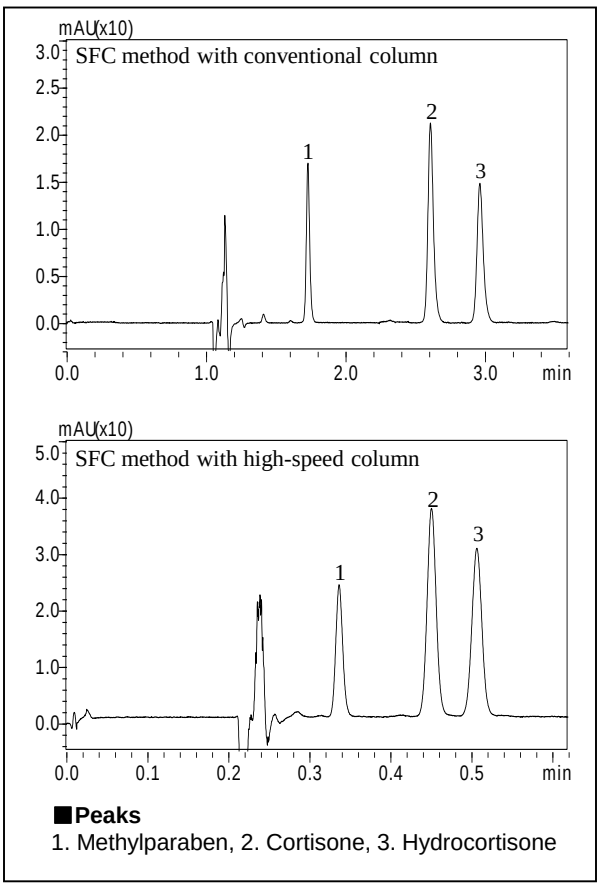

Fig.2: Chromatogram of system suitability solution (Upper: SFC method with conventional column, lower: lower: SFC method with high-speed column)

Table 5: System suitability results

System suitability requirements		CLC-SIL Nexera UC	XR-SIL Nexera UC
USP resolution between cortisone acetate and hydrocortisone acetate	≥ 2.2	4.64	2.61
Relative standard deviation for cortisone acetate	≤ 2.0 %	Rt 0.041 %	Rt 0.336 %
		Area 0.327 %	Area 0.563 %
Relative standard deviation for hydrocortisone acetate	≤ 2.0 %	Rt 0.052 %	Rt 0.331 %
		Area 0.448 %	Area 0.405 %

■ Reduction of Analysis Time and Cost

Fig.3 shows the comparison of analysis time and cost between the USP method and the SFC method with conventional column and high-speed column. By transferring the original USP method to an SFC method with conventional column, analysis time and cost was shortened by a factor of 2.4 and 6.5, respectively. By transferring the original USP method to an SFC method with high-speed column, analysis time and cost was shortened by a factor of 14 and 38, respectively. These results shows that the Nexera UC enabled significant reduction of analysis time and cost without sacrificing quality of analyses.

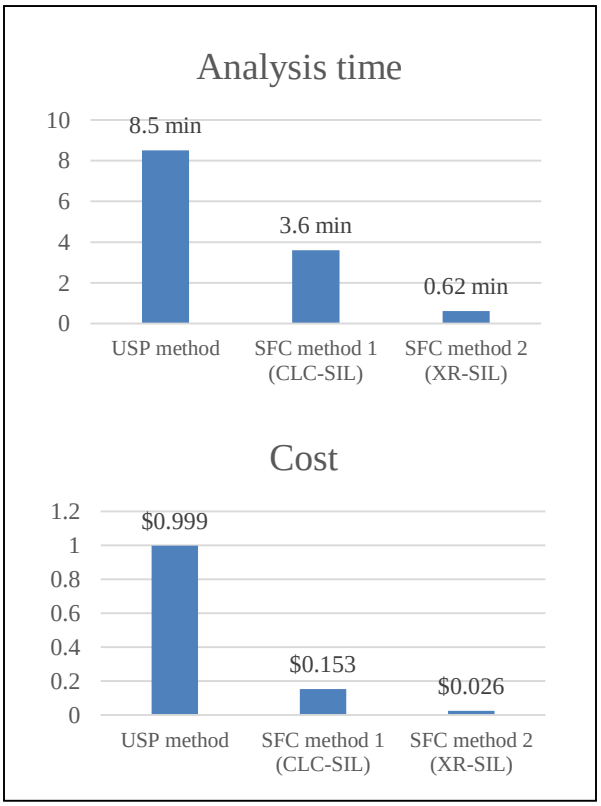

Fig. 3: Comparison of analysis time and cost

■ Reference

USP Monograph, Cortisone Acetate Tablets, USP 37-NF 32, Second supplement

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Shimadzu Corporation
www.shimadzu.com/an/