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Transfer of USP normal phase method for the analysis of cortisone acetate to the Nexera UC SFC system

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Introduction

The United States Pharmacopeia (USP) monographs are widely referenced to ensure the quality of drug substances. The USP monographs offer reliable and robust methods. However, some of these methods, especially those using normal phase HPLC, require the use of harmful, even toxic solvents. Naturally, laboratories are looking to reduce the use of such solvents for health and safety, as well as cost reasons. 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 SFC with a conventional HPLC column, Shim-pack CLC-SIL, and also a UHPLC phase, Shim-pack XR-SIL using the Nexera UC SFC system.

Figure 1: Nexera UC SFC-UV system

Method transfer to SFC

Table 1: Analytical conditions of the USP normal phase HPLC method

System	: Prominence
Column	: Shim-pack CLC-SIL (250 mm x 4.6 mm, 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 temperature	: Room temperature
Injection volume	: 15 µl
Detection	: UV 254 nm
Flow cell	: High-pressure cell for SFC

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The USP monograph for cortisone acetate tablet analysis specifies a 250 mm x 4.6 mm 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.

Table 2: Analytical conditions for SFC method (I)

System	: Nexera UC
Column	: Shim-pack CLC-SIL (250 mm x 4.6 mm, 5 µm)
Mobile phase	: A: CO_2 (85 %)
	B: Methanol (15 %)
Flow rate	: 3.0 ml/min
Column temperature	: 40 °C
Injection volume	: 2 µl
Detection	: UV 254 nm
Flow cell	: High-pressure cell for SFC

Figure 2 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) from a) the USP method and b) 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.

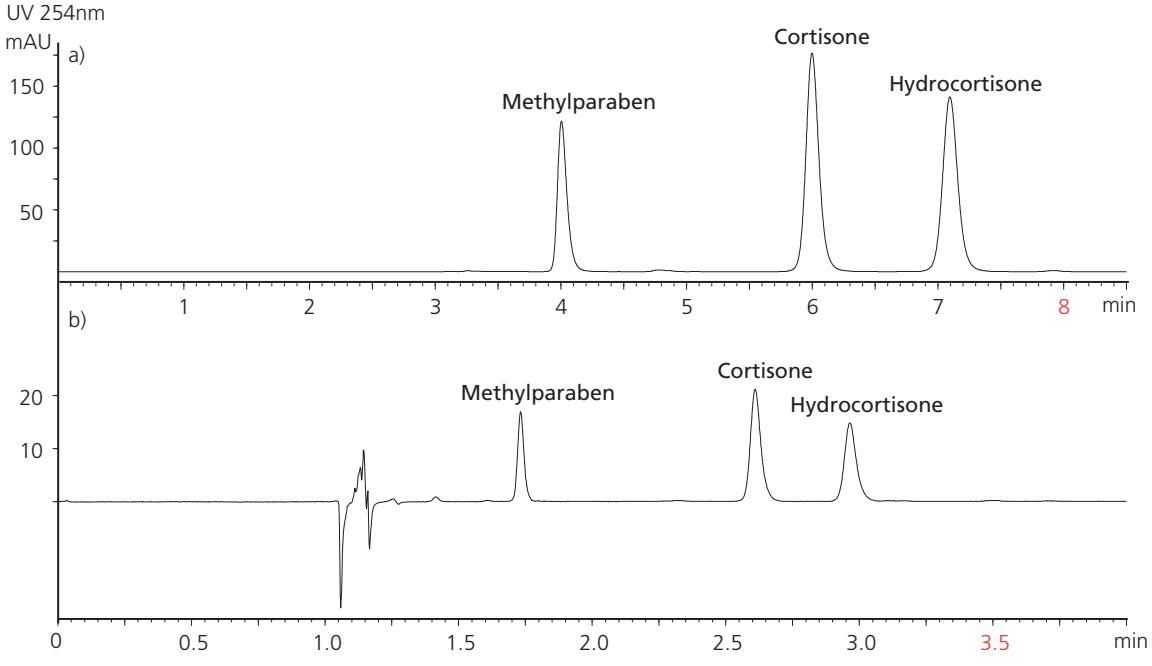

Figure 2: Chromatogram of system suitability solution with a) the USP method and b) the SFC method with conventional column

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Table 3: Results of system suitability test

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 hydrocortsione acetate	≤ 2.0 %	Rt.: 0.078 %	Rt.: 0.052 %
		Area: 0.142 %	Area: 0.448 %

Method Transfer to UHPLC column

An UHPLC column with small particles was used on the Nexera UC system to further shorten the run time. Table 4 shows the analytical conditions. Figure 3 shows the comparison of chromatograms from SFC method with conventional column (Shim-pack CLC-SIL) and UHPLC column (Shim-pack XR-SIL). The runtime was decreased 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 UHPLC column satisfies all of the criteria for the system suitability requirements in the USP-NF.

Table 4: Analytical conditions for SFC method (II)

System	: Nexera UC
Column	: Shim-pack XR-SIL (100 mm x 3.0 mm, 2.2 µm)
Mobile phase	: A: CO_2 (85 %)
	B: Methanol (15 %)
Flow rate	: 3.0 ml/min
Column temperature	: 40 °C
Injection volume	: 1 µl
Detection	: UV 254 nm
Flow cell	: High-pressure cell for SFC

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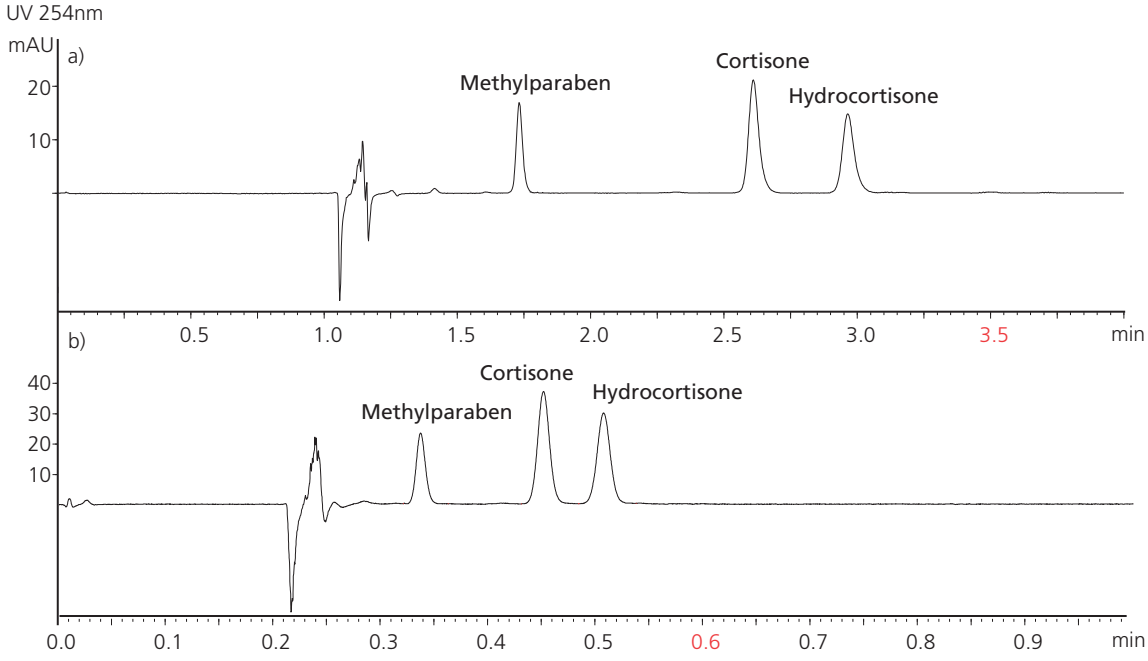

Figure3: Chromatogram of system suitability solution with a) SFC method with conventional column and b) SFC method with UHPLC column

Table 5: Results of system suitability test

System suitability requirements		CLC-SIL Nexera UC	CLC-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 hydrocortsione acetate	≤ 2.0 %	Rt.: 0.052 %	Rt.: 0.331 %
		Area: 0.448 %	Area: 0.405 %

Results

Figure 4 shows the comparison of analysis time and cost between the USP method and the SFC method with conventional column and UHPLC 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 UHPLC column, analysis time and cost was shortened by a factor of 14 and 38, respectively. These results show that the Nexera UC enabled significant reduction of analysis time and cost without sacrificing quality of analyses.

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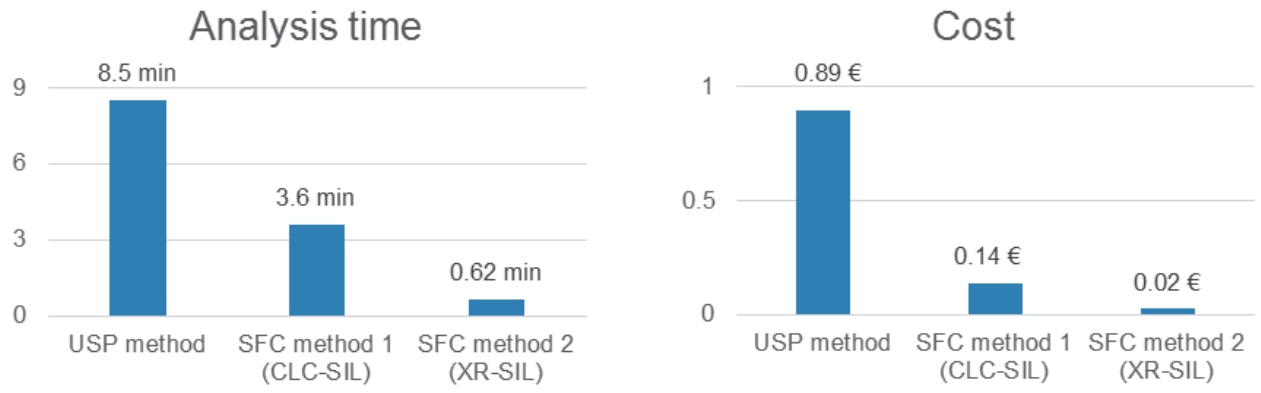

Figure 4: Comparison of time and cost using the conventional and alternative SFC method

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