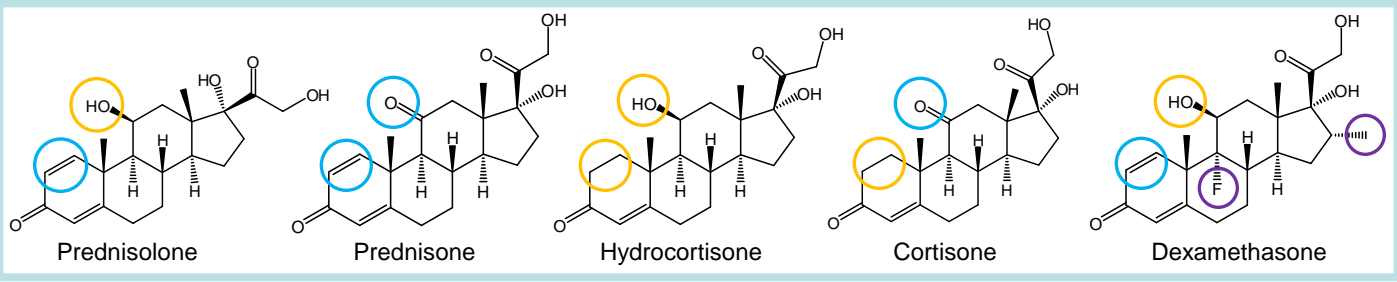


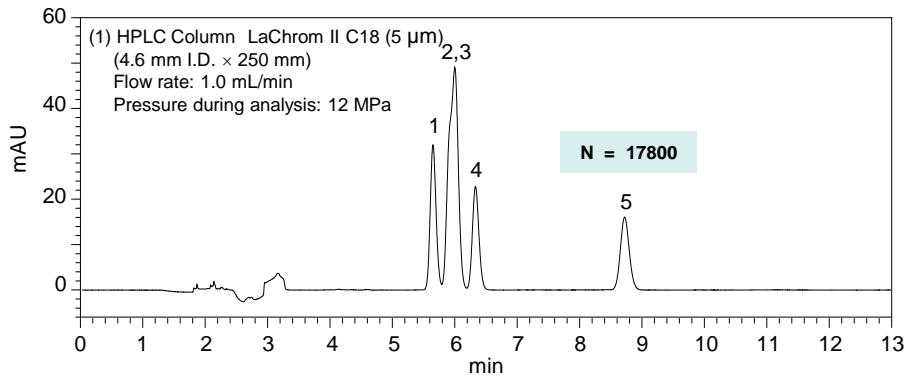
High Resolution Analysis of Steroids by UHPLC (Comparison of Organic Solvents) AS/LC-035

Steroid is a general term used for substances having the basic skeleton comprised of three 6-membered rings and one 5-membered ring. Many of them are bioactive compounds and widely used for pharmaceutical products, etc. As these compounds are similar in the structure, they are sometimes used as the model samples to show the separation by LC.

This time, by using a high resolution column for UHPLC (LaChromUltra II C18 (3.0 mm I.D. × 250 mm, 1.9 μm)) and a column for HPLC (5 μm), the separations of five components with the steroid skeleton were compared. The comparison was also made by using acetonitrile and methanol as the eluent. Hitachi Ultra High Performance Liquid Chromatograph, ChromasterUltra Rs, was used.



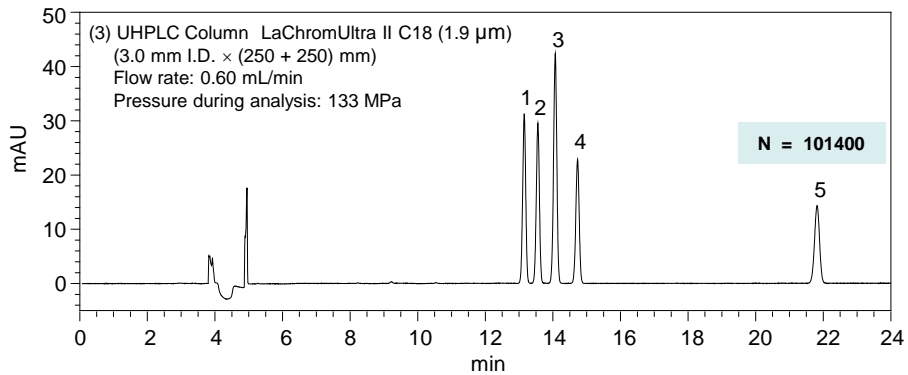
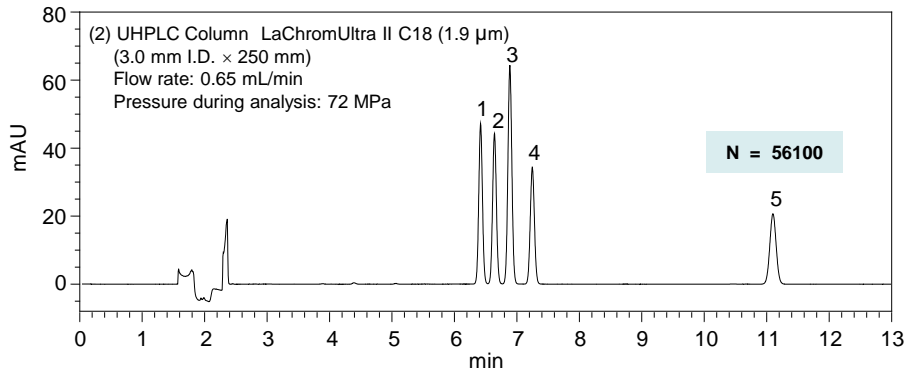
Analysis Example of 5 Steroids (10 mg/L each) (By Using Acetonitrile)



<Analytical Conditions>
 Column : (1) LaChrom II C18 (5 μm)
 4.6 mm I.D. × 250 mm
 (2) LaChromUltra II C18 (1.9 μm)
 3.0 mm I.D. × 250 mm
 (3) LaChromUltra II C18 (1.9 μm)
 3.0 mm I.D. × (250 + 250) mm
 Eluent : Water / Acetonitrile = 60 / 40 (v/v)
 Flow rate : (1) 1.0 mL/min
 (2) 0.65 mL/min
 (3) 0.60 mL/min
 Column temperature : 40°C
 Detection wavelength : UV 254 nm(DAD)
 Injection vol. : (1) 10 μL, (2)(3) 5 μL

<Steroid Standard Samples>
 1. Prednisolone
 2. Prednisone
 3. Hydrocortisone
 4. Cortisone
 5. Dexamethasone

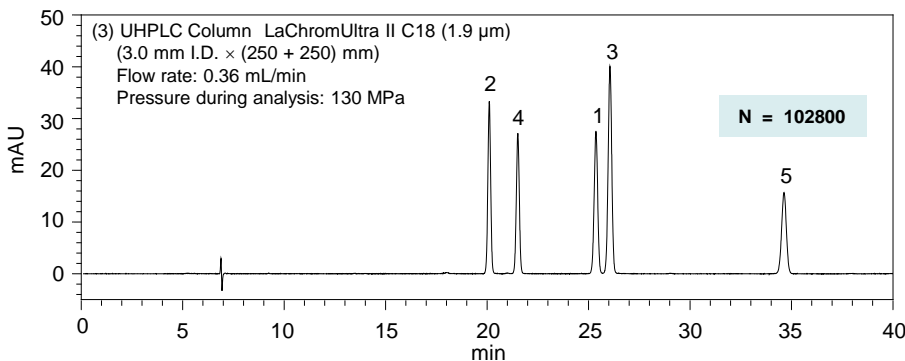
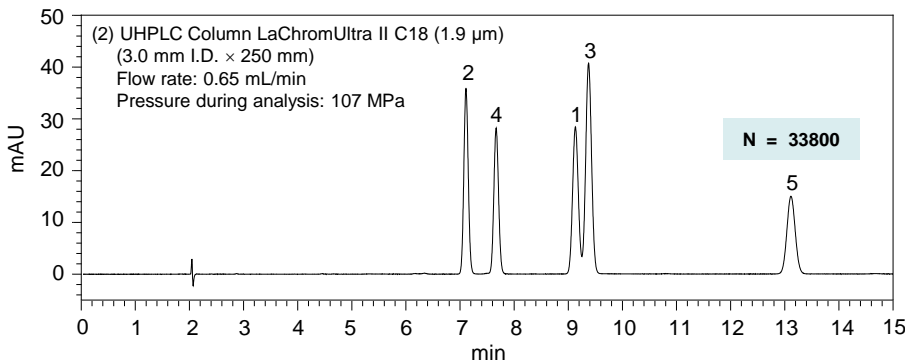
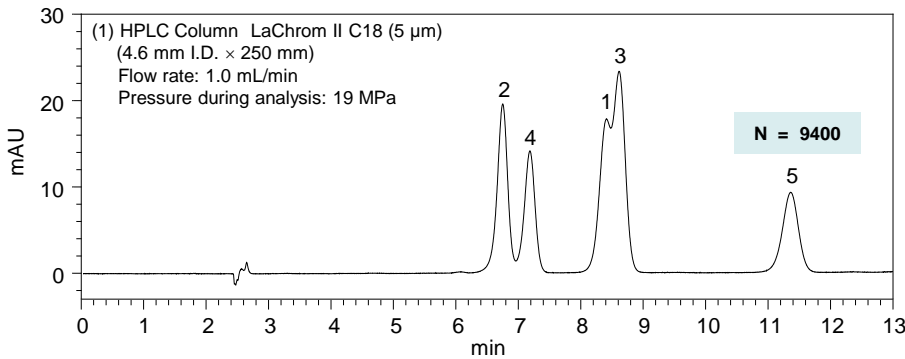
<Sample Preparation Method>
 The standard solution was prepared with methanol to have a concentration of 100 mg/L. After preparing the mixed standard solution with methanol to have a concentration of 10 mg/L, the solution was filtered through a 0.2 μm filter.



Standard sample	Resolution		
	(1) HPLC column	(2) UHPLC column	(3) 2 × UHPLC columns
1-2	—	2.0	2.4
2-3	—	2.1	2.9
3-4	—	3.0	3.6
4-5	10.5	24.9	30.9

A mixed solution of acetonitrile and water was used as the eluent.
 (1) 2. Prednisone and 3. Hydrocortisone cannot be separated by a HPLC column. (2) However, by using an UHPLC column, the separation for this part can be improved and it was possible to obtain a good resolution for the five components. In addition, by serially connecting two UHPLC columns, the number of the theoretical plates is increased and the resolution is improved although the analysis time is doubled.

Analysis Example of 5 Steroids (10 mg/L each) (By Using Methanol)



<Analytical Conditions>

Column : (1) LaChrom II C18 (5 μm)
 4.6 mm I.D. × 250 mm
 (2) LaChromUltra II C18 (1.9 μm)
 3.0 mm I.D. × 250 mm
 (3) LaChromUltra II C18 (1.9 μm)
 3.0 mm I.D. × (250 + 250) mm
 Eluent : Water/Methanol = 40 / 60 (v/v)
 Flow rate : (1) 1.0 mL/min
 (2) 0.65 mL/min
 (3) 0.36 mL/min
 Column temperature : 40°C
 Detection wavelength : UV 254 nm(DAD)
 Injection vol. : (1) 10 μL, (2)(3) 5 μL

<Steroid Standard Samples>

1. Prednisolone
2. Prednisone
3. Hydrocortisone
4. Cortisone
5. Dexamethasone

<Sample Preparation Method>

The standard solution was prepared with methanol to have a concentration of 100 mg/L. After preparing the mixed standard solution with methanol to have a concentration of 10 mg/L, the solution was filtered through a 0.2 μm filter.

Standard sample	Resolution		
	(1) HPLC column	(2) UHPLC column	(3) 2 × UHPLC columns
2-4	1.4	3.5	5.3
4-1	—	8.1	13.0
1-3	—	1.2	2.0
3-5	—	15.4	22.6

A mixed solution of methanol and water was used as the eluent.

(1) 1. Prednisolone and 3. Hydrocortisone cannot be separated by a HPLC column. (2) While the separation for this part can be improved by using an UHPLC column, it is still not sufficient. (3) By serially connecting two columns, the separation at the baseline was achieved and a good resolution was obtained for all five components.

It is shown that the elution order is different for acetonitrile and methanol.

Acetonitrile (1) → (2) → (3) → (4) → (5)

Methanol (2) → (4) → (1) → (3) → (5)

This is due to the properties of the organic solvents. Acetonitrile is an aprotic solvent while methanol is a protic solvent. It is known that the elution order changes as there is a different interaction with a target component.

When studying the elution conditions, it is sometimes useful to study the separation by using methanol rather than acetonitrile and the use of ChromasterUltra Rs system with LaChromUltra II C18 column, with the pressure resistance of 140 MPa, is effective.

Main system configuration: ChromasterUltra Rs DAD system
 (6170 Binary Pump, 6270 Autosampler, 6310 Column Oven, 6430 Diode Array Detector, Organizer)

NOTE: These data are an example of measurement; the individual values cannot be guaranteed.