

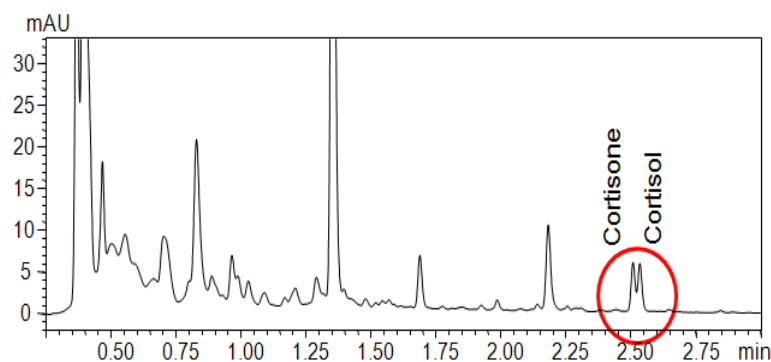
# Nexera Application Data Sheet No. 6

## Use of Methanol Solvent for Separation

In liquid chromatography acetonitrile is often a mobile phase ingredient of choice because of its elution strength in reverse phase analysis, low viscosity, low absorption in a wide range of the UV spectrum, and relatively low cost. However, in order to improve peak selectivity, researchers sometimes need to look for alternative solvents. Due to a wide pressure range of up to 130 MPa Nexera affords unlimited flexibility in choosing the ideal composition of the mobile phase even utilizing such viscous solvents as methanol-water that significantly increase the back pressure.

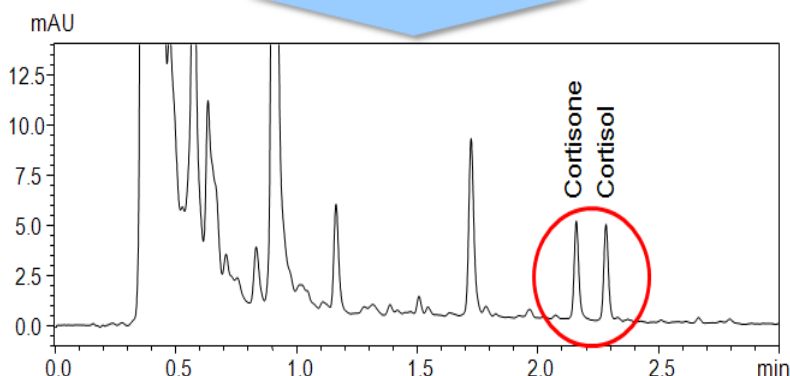
### Improved Separation of Cortisone and Cortisol

The chromatograms below show the analysis of cortisone and cortisol in urine (each 5  $\mu\text{g/mL}$ , spiked) first using mobile phase with acetonitrile then with methanol. Methanol mobile phase improved the peak resolution between cortisone and cortisol.



Column	: ODS (2.1 mm I.D. x 100 mm, 1.8 $\mu\text{m}$ )
Pressure	: 69 MPa
Mobile Phase	: A : 0.1% Formic acid in Water B : Acetonitrile
Gradient	: B 10% $\rightarrow$ 60% (3.5 min)
Flow Rate	: 0.6 mL/min
Column Temp.	: 40 $^{\circ}\text{C}$
Detection	: UV 245 nm

Methanol



Column	: ODS (2.1 mm I.D. x 100 mm, 1.8 $\mu\text{m}$ )
Pressure	: 108 MPa
Mobile Phase	: A : 0.1% Formic acid in Water B : Methanol
Gradient	: B 30% $\rightarrow$ 90% (3.0 min)
Flow Rate	: 0.6 mL/min
Column Temp.	: 40 $^{\circ}\text{C}$
Detection	: UV 245 nm