

Nexera Application Data Sheet No.15

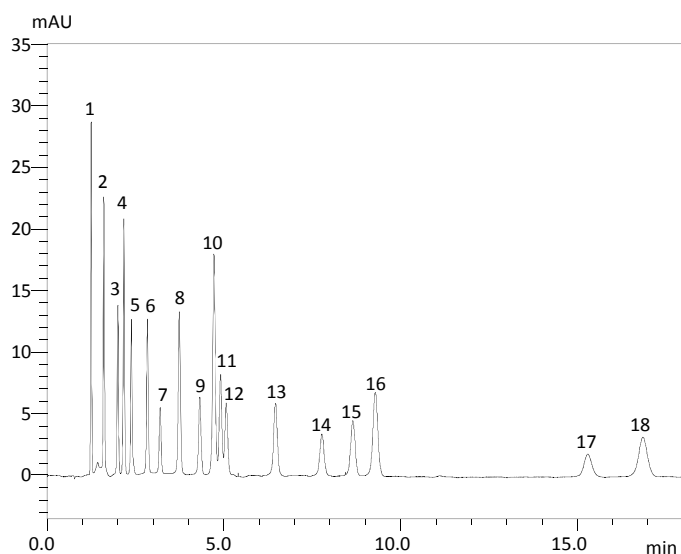
Ultrahigh Separation Analysis of Nucleotides

Nucleotides, building blocks of DNA and RNA, are compounds where phosphate(s) bond to a nucleoside that is composed of a nucleobase and sugar. In general, adenine (A), cytosine (C), guanine (G), thymine (T), and uracil (U) are examples of nucleobases, and ribose and deoxyribose are examples of sugar. There are many other types of nucleotides depending on the number of bonding phosphates (1 to 3).

For high-separation analysis of these components, a long column with a particulate packing material can be used. However, both the UHPLC unit and column need to have a high pressure-resistance. This document introduces an example of ultrahigh separation analysis performed on nucleotides using Nexera, which is resistant to 130 MPa, and a Shim-pack XR-ODS III column (particle size 2.2 μm), resistant to 100 MPa.

Batch analysis of 18 nucleotides

18 nucleotide standard mixtures (about 50 mg/L each) were separated using a 200 mm long Shim-pack XR-ODS III column (particle size 2.2 μm). Since isocratic elution was used, one analysis cycle could be performed in 18 minutes. In the analytical conditions, the system pressure under load was 78 MPa.



Column	: Shim-pack XR-ODS III (200 mmL. x 2.0 mmI.D., 2.2 μm)
Mobile phase	: A: 100 mmol/L Phosphoric acid/ 150 mmol/L Triethylamine aq. B: Mobile phase A/Acetonitrile=9/1(v/v) A/B = 98/2(v/v)
Flow rate	: 0.6 mL/min
Column temp.	: 50 $^{\circ}\text{C}$
Injection volume	: 1 μL
Detection	: UV 260 nm
Flow cell	: Semi-micro cell
Pressure	: 78 MPa

Peaks:

- | | |
|-------------------------------------|--------------------------------------|
| 1. CMP (Cytidine 5'-monophosphate) | 10. AMP (Adenosine 5'-monophosphate) |
| 2. UMP (Uridine 5'-monophosphate) | 11. TMP (Thymidine 5'-monophosphate) |
| 3. CDP (Cytidine 5'-diphosphate) | 12. UTP (Uridine 5'-triphosphate) |
| 4. GMP (Guanosine 5'-monophosphate) | 13. GTP (Guanosine 5'-triphosphate) |
| 5. IMP (Inosine 5'-monophosphate) | 14. ITP (Inosine 5'-triphosphate) |
| 6. UDP (Uridine 5'-diphosphate) | 15. TDP (Thymidine 5'-diphosphate) |
| 7. CTP (Cytidine 5'-triphosphate) | 16. ADP (Adenosine 5'-diphosphate) |
| 8. GDP (Guanosine 5'-diphosphate) | 17. TTP (Thymidine 5'-triphosphate) |
| 9. IDP (Inosine 5'-diphosphate) | 18. ATP (Adenosine 5'-triphosphate) |