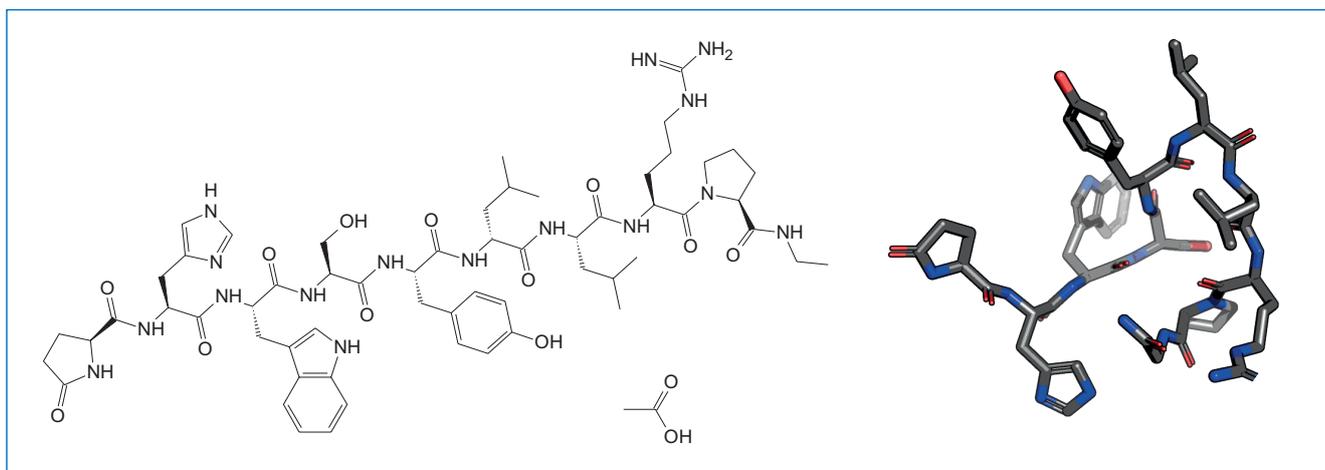


Analysis of leuprorelin acetate using YMC-Triart Bio C18

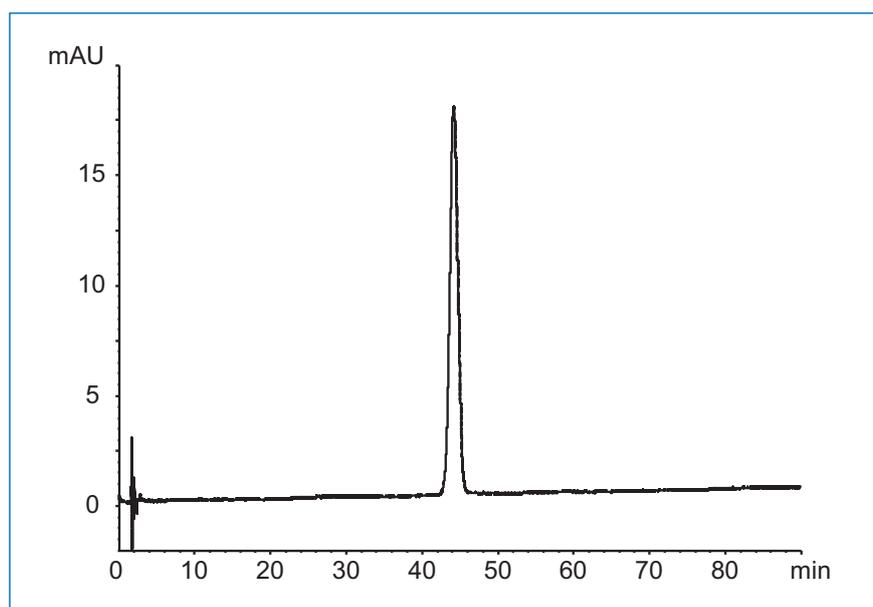
The peptide leuprorelin is a synthetic analogue of gonadotropin-releasing hormone (GnRH) and consists of 9 amino acids (MW: 1.2 kDa). It decreases production of gonadotropin and therefore decreases testosterone and estradiol levels in blood. It is used in treatment of hormone-responsive cancers such as prostate cancer and breast cancer and different hormone related diseases. Unlike the endogenous decapeptide GnRH, leuprorelin contains a single D-amino acid (D-leucyl) residue.



Leuprorelin acetate

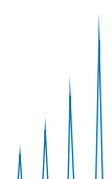
The Japanese Pharmacopeia (JP) describes a method to analyse leuprorelin and its degradation products using a C18 column and a fixed retention time range.

(A) Leuprorelin acetate (0.05 mg/mL)



YMC-Triart Bio C18 has a lower surface area and provides a lower hydrophobicity due to its 300 Å pore size. By applying a flow rate of 0.75 mL/min the retention time criterion (41–49 min) of the JP is matched.

By using YMC-Triart Bio C18 the separation could be speeded up by applying a higher flow rate of e.g. 1 mL/min which results in a retention time of about 34 min.





YMC-Triart Bio C18 is also able to separate leuprorelin from its degradation products caused by alkaline degradation as described below.

(B) Leuprorelin acetate alkaline degradation analysis

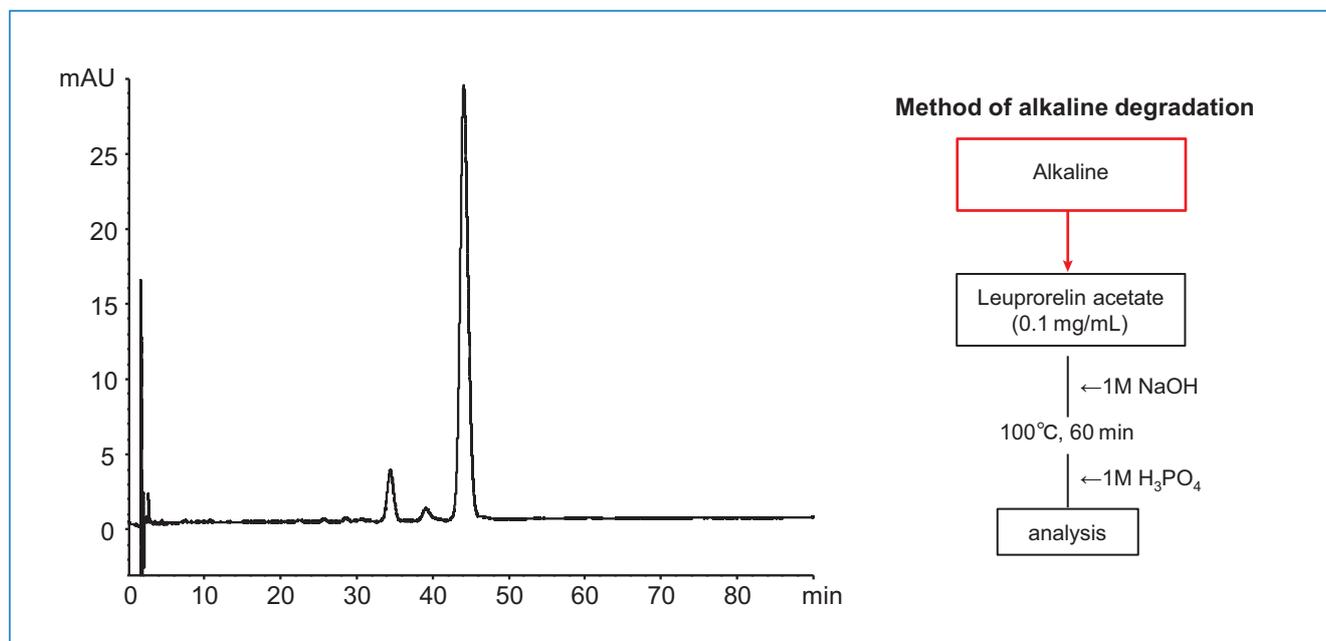


Table 1: Chromatographic conditions

Column:	YMC-Triart Bio C18 (3µm, 30nm) 100 x 4.6mm ID
Part No:	TA30S03-1046PTH
Eluent:	buffer (pH 3.0)*/acetonitrile/1-propanol (85/9/6) *Dissolve 15.2g of triethylamine in 800mL of water and adjust pH 3.0 with H ₃ PO ₄ , and add water to make 1000mL.
Flow rate:	0.75 mL/min
Temperature:	25 °C
Detection:	UV at 220 nm
Injection:	20 µL

The flow rate originally suggested by the JP is 1–1.5 mL/min. By using YMC-Triart Bio C18 the analysis only requires a flow rate of 0.75 mL/min to elute leuprorelin in the middle of the required retention time frame. Therefore, this application note is only a reference to the JP monograph, and the analysis time can be reduced.

Reference

The Japanese Pharmacopoeia 17th Edition, Leuprorelin Acetate / Official Monographs

