# Preparative Purification of Leuprorelin by HPLC Applying a "Saw-Tooth" Gradient

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## Key Words

Column liquid chromatography
Preparative-scale separations
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## Summary

Recently we reported on the development of a new semi-preparative HPLC technique for the purification of leuprorelin [1]. This new method, referred to as a "saw-tooth technique" utilizes two successive gradients of two different eluent systems on the same column. Here we show our new results on extending the purification of leuprorelin from the semi-preparative 0.1–2 g) to the preparative (3–25 g) scale.

## Introduction

In the pharmaceutical industry synthetic peptides purified by HPLC are often applied directly as medicaments. Efficient analytical HPLC techniques developed may not be able to be scaled up on account of the demand choosing appropriate counterions for pharmaceutical use. It is thereforde, necessary to develop a totally different separation technique for semi-preparative and preparative HPLC purifications.

Leuprorelin (Glp-His-Trp-Ser-Tyr-D-Leu-Leu-Arg-Pro-NHEt) is a LHRH analogue and was synthesized by classical methods in solution. The crude product contained 23–28 % impurities of peptidic nature. The aim of the work presented here was to obtain leuprorelin as the acetate salt, exceeding 98 % purity.

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## Experimental

A Gilson HPLC system was used, consisting of two pumps (Model 305 and 306 with different pump heads, Model 811C dynamic mixer, Model 806 manometric module) and a Shimadzu SPD-10A UV detector. C-18 columns of 10 mm (VYDAC Prot & pept. 218TP152010) or 22 mm (WHATMAN Partisil ODS-3, 10 $\mu$m, 250 mm length, or 500 mm length) diameter were used for the separation in the 0.1 g–2 g range. 50 mm diameter columns (VYDAC Prot & pept. 218TP152050, 250 mm length or YMC-Pack ODS-AQ, S-10/20, 120A, 500 mm length) were used to separate 3–25 g of the crude synthetic peptide.

## Results and Discussion

In preliminary experiments several eluent systems were screened in order to optimize the separation. These systems contained either pure water, acetic acid, trifluoroacetic acid, phosphoric acid, hydrochloric acid, ammonium-hydrogen-carbonate, or sodium dihydogenphosphate. Methanol, ethanol, 2-propanol or acetonitrile were used as organic modifier. In summary, none of these experiments based on simple gradient chromatography gave satisfactory results. Nevertheless some eluent systems were nearly efficient on a preparative, but not at all on an analytical scale.

In the light of the se preliminary experiments we decided to combine the advantages of two different eluent systems. The preparative HPLC process of double gradients for the purification of leuprorelin acetate salt consisted of three steps. At first, most of the impurities were removed with a water/acetonitrile gradient, while leuprorelin was retained in the upper part of the column. When the first gradient was stopped, the column was washed out with 0.2 % acetic acid in water and finally a second acetonitrile gradient was started with an eluent containing 0.2 % acetic acid. Leuprorelin was eluted only during the second gradient. We proposed the term "saw-tooth gradient" for this techique [1].



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During purification by this gradient technique the anion form of crude peptide salt should be taken into account. By extending the purification from the semi-preparative to preparative scale, using a saw-tooth gradient, leuprorelin also appeared during the first gradient step. The reason of this was that after synthesis, the crude peptide was in chloride form. After a preliminary ion exchange to acetate form, leuprorelin eluted only in the second gradient step.

## Conclusions

A successful combination of two different gradient systems, the so-called "saw-tooth" double gradient process, resulted in leuprorelin sufficiently pure for human purposes. We stress that due to the special demands, preparative chromatographic separations are often accomplished under non-ideal conditions.

## Reference

[1] *A.Rill, O.Nyéki, M.Gazdag, I.Schon*, in peptides 1998 (Proceedings of the Twenty-Fifth European Peptide Symposium., edited by S. Bajusz, F.Hudecz, Akadémiai kiadó, Budapest) 324 (1999)

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