Application Note

No. 64

HPLC of Peptides at Lowered Temperatures

Steel Column 8x80 TESSEK Separon EDMA 40 10 μm

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Chromatographic Conditions

Column:	Steel column 8x80 mm TESSEK Separon EDMA 40 10 μm
Eluent:	Acetonitrile-methanol-0.1% trifluoroacetic acid 7:46.5:46.5
Flow rate:	1 ml/min
Detection:	UV at 222 nm
Sensitivity:	0.1 AUFS
Injection:	20 μl

Fig. 1 Sample of CCK-pentapeptide on Vydac C18 (25x0.4 cm) at different temperatures.

Fig. 2 The same sample on TESSEK Separon EDMA 40 .

Fig. 3 Mixture of [D- and [L-Tic^2]OXT on EDMA 40 at different temperatures.

Fig.1

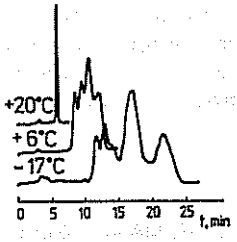


Fig.2

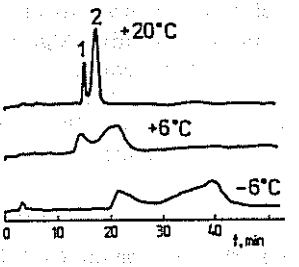
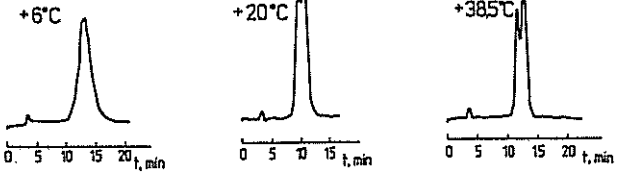


Fig.3



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Introduction

The effect of increased temperature on the HPLC separation was studied in several papers. Effect of decreased temperature was studied much less, since it may lead to substantial decrease of the column separation power. However, in the cases when fast interconversion between two or more forms of analysed molecule is to be expected, decrease of temperature may lead to the separation of these forms.

Sample preparation

HPLC purified peptide Gly-Trp-MeNle-Asp-Phe-NH_2 was analysed on a Vydac C18 column (Separations Group, Hesperia, USA), where it has shown only one sharp symmetrical peak when analysed at normal temperature. The same sample analysed on EDMA 40 column have shown two peaks, which were separated preparatively. Further analysis have shown that the first peak did not contain the required peptide.

Results

Decrease of the temperature led in the case of Vydac C18 column to the separation of five components from the peptide sample (Figure 1). Peaks separated on TESSEK Separon EDMA 40 column (Figure 2) were shown to be a mixture of impurities (peak 1) and mixture of cis and trans conformers of studied peptide (peak 2). Conformer mixture can be separated on Vydac C18 column at decreased temperature, but not on EDMA 40 column (Figure 2). The effect of the temperature on the separation was further studied in the case of [L- and [D-Tic^2]OXT, which are normally eluted very close to each other. Effect of temperature is observable on Figure 3.

Conclusions

Vydac C18, which is considered to be the optimal carrier for peptide separtion, was shown to give an erroneous result in the case of relatively simple pentapeptide. Very short column of TESSEK Separon EDMA 40 was able to show and separate impurities from the sample which looked homogeneous on Vydac C18. The decreased temperature decreased the efficiency of both tested columns, especially in the case of EDMA 40. The increased temperature can improve separation of closely eluted compounds.

Ordering Information

Research prod. Steel column 8x80 TESSEK Separon EDMA 40 10 μm