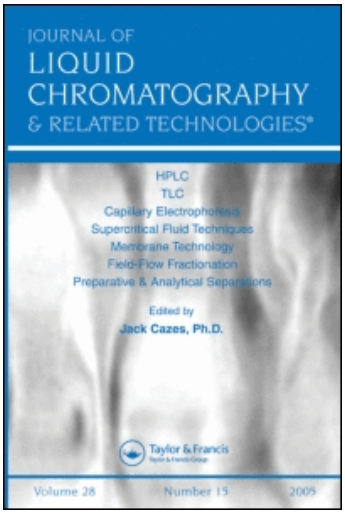

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Separation of Selected Dipeptides by High Performance Liquid Chromatography

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SEPARATION OF SELECTED DIPEPTIDES BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

The use of β-cyclodextoin bonded column for the separation of a selected group of dipeptides was explored. The results show that the separation of the dipeptides using a methanol/water (16:84) as the mobile phase and the above column is possible.

INTRODUCTION

Peptides are composed of two or several, which may be the same or different, amino acids joined by an amide bond, where the carboxy group of one amino acid is bonded to the amino group of the next amino acid by an amide. A dipeptide is made of two amino acid molecules.

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This study will deal with the separation of a selected group of dipeptides by high performance liquid chromatography, using a β-cyclodextrin bonded column. The β-cyclodextrin unit consists of 7 glucose molecules forming a truncated cone shape, having a hydrophilic surface and a hydrophobic interior cavity. The theory of separation using the β-cyclodextrin columns have been discussed by Armstrong and his co-workers (1).

The columns have been used for the separation of some D- and L-amino acids (2) and other molecules (3). The present study extends the use of the β-cyclodextrin column for the separation of dipeptides.

EXPERIMENTAL

Materials

The dipeptides used in this study were purchased from United States Biochemical Corporation (Cleveland, OH) and used without further purification. Methanol was glass distilled UV grade (Burdick and Jackson, Muskegon, MI). The β-cyclodextrin column (Cyclobond I) was purchased from Advanced Separations Technology, Inc. (Whippany, NJ).

Apparatus

A Hewlett-Packard Model 1090 Liquid Chromatograph equipped with a photodiode array detector, an automatic injector, a strip chart recorder, a Hewlett-Packard Model 3392A integrator and a Hewlett-Packard Model 85 computer/controller was used. A cyclobond I column, 250 mm x 4.6 mm, packed with β-cyclodextrin bonded to 5 nm irregular silica gel particles was used. 5 ml of solution was injected and the

absorption was monitored at 254 nm. The mobile phase was 16% methanol/water. The mobile phase was filtered and degassed before use and maintained under helium throughout the experiment.

RESULTS AND DISCUSSION

The dipeptides selected for this study are those which had a π-bond, and which would be easily detected at 254 nm. Ten of these dipeptides were used to evaluate the utility of the cyclodextrin column for HPLC separation of peptides. Two pairs of these ten dipeptides are made each of the same amino acids, glycyl-phenylalanine and phenylalanyl-glycine and tyrosyl-phenylalanine and phenylalanyl-tyrosine. The results are given in the following table.

The results show that the dipeptides can be separated using a cyclobond I column and an isocratic mobile phase of 16% methanol/water. The use of gradient elution (work in progress) should improve the resolution.

TABLE 1

Results of the Separation (Rt) of Dipeptides Using a Cyclobond I Column and a Mobile Phase of 16% Methanol/Water

Dipeptide	Rt (min)
glycyl-phenylalanine	6.63
tyrosyl-phenylalanine	8.35
tyrosyl-isoleucine	11.91
phenylalanyl-leucine	17.38
phenylalanyl-glycine	7.76
tryptophyl-alanine	9.51
tryptophyl-leucine	20.52
tryptophyl-tyrosine	5.66 (87%)* 12.43 (13%)*
tyrosil-tyrosine	3.14 (14%)* 6.74 (86%)
phenylalanyl-tyrosine	10.13 (40%)* 29.5 (60%)

*It is assumed that the smaller peak is an impurity.

The results show that good resolution was obtained for glycyl-phenylalanine (Rt = 6.63), tyrosyl-phenylalanine (Rt = 8.35), phenylalanyl-leucine (Rt = 17.38), phenylalanyl-tyrosine (Rt = 29.5) and phenylalanyl-glycine (Rt = 7.76), although they vary by one amino-acid. It was also possible to separate two pairs of dipeptides which are made each of the same amino acids; phenylalanyl-glycine (Rt = 7.76) and glycyl-phenylalanine (Rt = 6.63), which were separated by more than one minute, and tyrosyl-phenylalanine (Rt = 8.35) and phenylalanyl-tyrosine (Rt = 29.5), which were separated by 21 minutes.

We are evaluating the use of the β-cyclodextrin column for the separation of other short- and long-chain peptides and hope to report those results shortly in this journal.

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