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Multi-column preparative reversed-phase sample displacement chromatography of peptides

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ABSTRACT

Preparative reversed-phase sample displacement chromatography (SDC) of peptides was examined utilizing a multi-column approach. The effects of various SDC run parameters (flow-rate, run time and sample load) on the distribution of a single purified peptide and a mixture of three synthetic peptides was examined. The peptides in the mixture were closely related in hydrophobicity and mixed in a 1:4:1 ratio designed to mimic a typical preparative separation problem frequently encountered in crude synthetic peptide mixtures, that is, where there exist both hydrophobic and hydrophilic synthetic impurities close to the product of interest.

Based on the results of these model systems, a SDC protocol was applied to the preparative purification of a crude synthetic peptide. The multi-column SDC approach provides rapid separations that are easy to employ because isocratic elution is utilized both in the separation process and in elution of the column segments. There is minimal fraction analysis, minimal use of organic solvents and increased utilization of the stationary phase such that the method involves considerably lower costs than traditional gradient-elution chromatography.

INTRODUCTION

The most common analytical method employed for reversed-phase chromatography (RPC) of peptides involves linear gradient elution (gradient-rate of 1% eluent B/min at a flow-rate of 1 ml/min), where eluent A is 0.05–0.1% (v/v) aqueous trifluoroacetic acid (TFA) and eluent B is 0.05–0.1% (v/v) TFA in acetonitrile [1,2]. The elution mode of RPC, however, is handicapped by relatively poor utilization of the stationary and mobile phases [3]. Thus, in order to obtain satisfactory yields and purities of peptides which are closely related in hydrophobicity, large-scale gradient-elution separations of peptides often require large, costly columns.

With the growing use of synthetic peptides in biochemistry, immunology and in the pharmaceutical and biotechnology industries, there is a need for easier and more reliable methods for purification of peptides. We report here a novel preparative method for preparative-scale reversed-phase purification of peptides on analytical columns termed sample displacement chromatography (SDC), which is characterized by the major separation process taking place in the absence of an organic modifier. SDC separations use the well-established general principles of displacement chromatography [3,4] without using a displacer [5–7].

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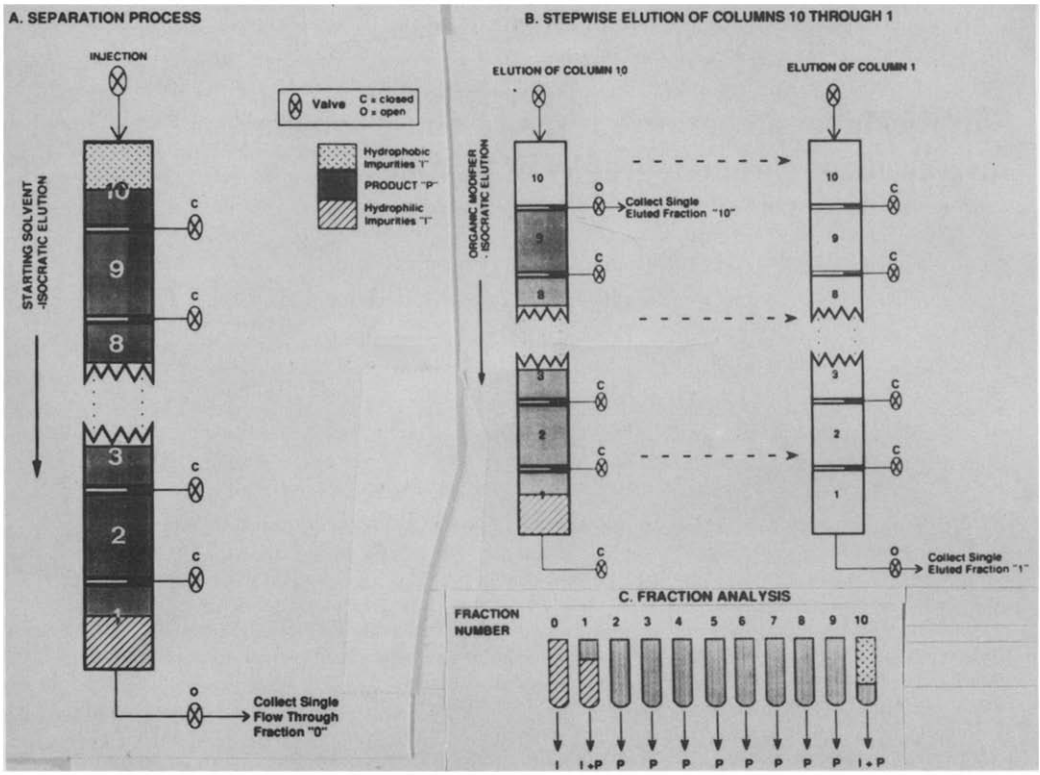

Fig. 1. Schematic representation of multi-column SDC. *Step A (separation process):* small reversed-phase column segments (ten in this case, numbered 1–10) have been connected in series. Column 1 is closest to the detector and column 10 closest to the injector. The sample mixture, containing the desired peptide product (*P*) as well as hydrophilic and hydrophobic impurities (*I*) is introduced by isocratic elution in the starting eluent. Isocratic elution in this eluent is then continued at a specified flow-rate and run time to complete the separation. Step A shows a representation of sample loading and the subsequent isocratic elution of a typical distribution of peptide components through the total column length following SDC. Purified peptide product (*P*) can be found in columns 2–9; hydrophobic impurities remain in column 10 and hydrophilic impurities in column 1. Hydrophilic impurities may also be displaced from the column into the breakthrough fraction 0. *Step B (stepwise elution of column segments):* each individual column segment is eluted to remove retained solute(s). This may be achieved either by isolating each column segment via a valve system (as shown) or by disconnecting all column segments prior to individual elution. The retained peptide components are removed from each column segment by an isocratic wash with an aqueous solution of organic modifier. The left schematic in step B represents the situation where only column 10 has been eluted to produce fraction 10; the right schematic represents the situation where all the columns have been individually eluted to produce fractions 1–10. Each column represents a fraction. Thus, for 10 column segments, there is a maximum of 11 fractions (10 columns plus the breakthrough fraction 0). *Step C (fraction analysis):* each tube number (1–10) corresponds to a fraction eluted from each column segment. Fraction 0 represents the breakthrough fraction containing only hydrophilic impurities displaced from the columns during SDC. The amounts of hydrophilic impurities/product (fraction 1), pure product (fractions 2–9) and hydrophobic impurities/product (fraction 10), represent the peptide components eluted from columns 1–10 (schematic in step A).

This paper aims to develop our understanding of the sample displacement process by examining the effect of various run parameters (flow-rate, sample load, run time) on the retention behaviour of peptides during SDC, and to introduce a multi-column approach to SDC, which promises to increase further the ease and efficiency of an already promising preparative approach to the separation of peptides (Fig. 1).

Principles of SDC utilizing a multi-column approach.

(1) The reversed-phase column consists of small column segments connected in series.

(2) The sample mixture containing the desired peptide product as well as hydrophobic and hydrophilic impurities is dissolved in the starting eluent (in this case, a 100% aqueous mobile phase, 0.05–0.1% aq. TFA), injected onto the column, and eluted isocratically at a specific flow-rate and run time to complete the separation. At high sample load, there is competition by the sample components for the adsorption sites on the hydrophobic stationary phase. The sample components act as their own displacers, with the more hydrophobic peptide components displacing the more hydrophilic components. Thus, the separation process can take place in the absence of organic modifier.

(3) At the end of the separation, the components are distributed on the column according to their relative hydrophobicities: the most hydrophilic near the column outlet and the most hydrophobic near the column inlet. The retained peptide components are removed quickly and efficiently from each column segment by an isocratic wash with an aqueous solution of organic modifier. The concentration of organic modifier need only be just above that required to elute the component of interest (gauged from an analytical gradient–elution run of the original sample mixture).

(4) This methodology is very simplistic, since no gradient elution is involved and the number of fractions for analysis is minimized, since each column represents a fraction. The method, unlike traditional gradient elution, maximizes sample load by making very efficient use of column capacity. In addition, the sample displacement process has high resolving power, leading to increased yields.

EXPERIMENTAL

Materials

High-performance liquid chromatographic (HPLC)-grade water and acetonitrile were obtained from J. T. Baker (Phillipsburg, NJ, USA). HPLC-grade TFA was obtained from Pierce (Rockford, IL, USA).

Peptides were synthesized either on an Applied Biosystems (Foster City, CA, USA) Model 430A peptide synthesizer, using the general procedure for solid-phase peptide synthesis described by Parker and Hodges [8] and Hodges *et al.* [9]. All three peptides used in this study were based on the sequence Ac–Arg–Gly–X–Y–Gly–Leu–Gly–Leu–Gly–Lys–amide, where positions X–Y were substituted with Ala–Gly (peptide S3), Val–Gly (peptide S4) or Val–Val (peptide S5). All peptides contained an N^{α}-acetylated N-terminal and a C-terminal amide.

Apparatus

The HPLC instrument consisted of a Varian Vista Series 5000 liquid chromatograph (Varian, Walnut Creek, CA, USA) coupled to an Hewlett-Packard (Avon-

dale, PA, USA) HP1040A detection system, HP9000 Series 300 computer, HP9133 disc drive, HP2225A Thinkjet printer and HP7440A plotter.

Column packings

Separations were carried out on two reversed-phase column packings: either (A) Rainin C_8 (11–12 μm particle size, 330 Å pore size; Rainin Instrument Company, Berkeley, CA, USA); or (B) Aquapore RP-300 C_8 (7 μm, 300 Å; Brownlee Labs., Santa Clara, CA, USA).

Run conditions

Preparative separations were carried out with 0.1% (v/v) aq. TFA as the mobile phase eluent.

Analytical separations were carried out by linear AB gradient elution, where eluent A was 0.1% (v/v) aq. TFA and eluent B was 0.1% TFA (v/v) in acetonitrile.

The absorbance of the peptides was detected at 210 nm.

RESULTS AND DISCUSSION

Unless stated otherwise, all of the preparative data presented here were produced on a multi-column setup, consisting of six 3 cm × 4.6 mm I.D. Rainin C_8 column segments (columns 1–6) in series. The numbering of the columns (or fractions) starts at the column segment closest to the detector. Thus, column 1 (fraction 1) is at the multi-column outlet, while column 6 (fraction 6) is at the inlet. The fraction marked 0 is the breakthrough fraction, consisting of components not retained by, or displaced from, the column.

Column equilibration, sample loading and the SDC runs were all carried out in 0.1% (v/v) aq. TFA.

Following each run, the individual column segments were eluted with 25% (v/v) aq. acetonitrile containing 0.1% (v/v) TFA and the resulting peptide solutions subjected to analysis using standard gradient elution (1% B/min at 1 ml/min) on the multi-column setup. It should be stressed that each column segment is a fraction, *i.e.*, for this six column setup, there is a maximum of seven fractions (six column segments plus the breakthrough fraction).

Multi-column SDC of a single peptide component

Before applying the multi-column SDC approach to peptide mixtures, the retention behaviour of a single peptide component under varying run parameters was investigated. SDC was applied under various flow-rates, sample loads and run times to a single synthetic decapeptide (S5).

As the flow-rate was increased, the peptide moved further down the total column length (Table I) indicating that flow-rate affects column capacity. A ten-fold decrease in flow-rate increased the sample capacity of the total column packing by about a third [six column segments at 2 ml/min (columns 1–6) to four column segments at 0.2 ml/min (columns 3–6)].

As run time was increased, there was a slight increase in the distance that the peptide moved down the total column length (Table I) [a four-fold increase in run time (36 min to 144 min) resulted in the peptide moving by one further column

TABLE I

EFFECT OF VARYING RUN PARAMETERS ON MULTI-COLUMN SDC OF A SINGLE DECAPEPTIDE

Results are presented as amount of peptide (mg) recovered from each fraction following SDC. Column: six 3 cm × 4.6 mm I.D. Rainin C_8 column segments (columns 1–6) in series. The numbering of the columns (or fractions) starts at the column segment closest to the detector. Thus, column 1 (fraction 1) is at the multi-column outlet, while column 6 (fraction 6) is at the inlet. The fraction marked 0 is the breakthrough fraction. Conditions: column equilibration, sample loading and the SDC runs were all carried out in 0.1% (v/v) aq. TFA. Fraction analysis: individual column segments were eluted with 25% (v/v) aq. acetonitrile containing 0.1% (v/v) TFA and samples of the resulting peptide solutions were subjected to linear AB gradient elution (1% B/min at 1 ml/min) on the multi-column setup. Absorbance at 210 nm. Effect of flow-rate: run time and sample load were 36 min and 25 mg, respectively. Effect of run time: flow-rate and sample load were 0.2 ml/min and 25 mg, respectively. Effect of sample load: flow-rate and run time were 0.2 ml/min and 36 min, respectively. The sequence of the synthetic peptide (S5) is shown in Experimental.

Fraction number	Effect of flow-rate (ml/min)			Effect of run time (min)			Effect of load (mg)		
	2	0.5	0.2	36	72	144	50	25	10
0	0	0	0	0	0	0	3.2	0	0
1	1.8	0	0	0	0	0	7.0	0	0
2	5.3	1.7	0	0	3.8	7.3	7.5	0	0
3	5.1	6.0	3.7	3.7	5.7	6.4	7.3	3.7	0
4	5.0	6.4	7.6	7.6	5.3	4.7	7.9	7.6	0
5	4.4	5.9	7.6	7.6	6.3	4.3	8.0	7.6	5.0
6	3.3	5.1	6.1	6.1	4.0	2.3	9.1	6.1	5.0

segment (column 3 to column 2)]. Thus, run time did not substantially affect column capacity.

The total column capacity was between 25 mg and 50 mg (Table I), since 25 mg did not exceed column capacity; whereas with a 50-mg load, there was peptide in the breakthrough fraction (3.2 mg in fraction 0 with the 50-mg load).

Multi-column SDC of a multiple-component mixture

An examination was carried out on the distribution of three peptide components through the length of the column packing, following multi-column SDC under varying run parameters of flow-rate, sample load and run time.

Fig. 2 shows an analytical run of the model three-decapeptide mixture (S3, S4 and S5) on the six-column C_8 setup.

The ratio of the peaks is approximately 1:4:1 (S3:S4:S5). The main component (S4) represents the desired product from a peptide synthesis. The smaller peaks represent more hydrophilic (earlier eluted, S3) and hydrophobic (later eluted, S5) peptide impurities. The peptide mixture was subjected to SDC as shown in Figs. 3–5 and Table II.

Effect of flow-rate. All three peptides moved further down the total column length with increasing flow-rate. At 2 ml/min (Table II), all of S3 has been displaced into the breakthrough fraction (fraction 0) and a significant portion ($\approx$40%) of S4, in addition to S3, has also been displaced from the column. Thus, at 2 ml/min, a sample load of 24 mg overloaded the column.

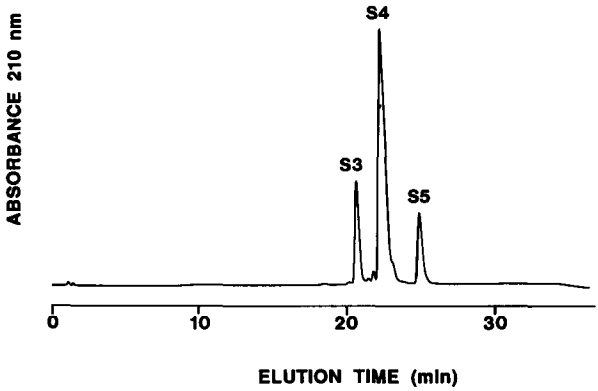

Fig. 2. Analytical RPC of a model three-decapeptide mixture. Column: six 3 cm × 4.6 mm I.D. Rainin C_8 column segments in series. Conditions: linear AB gradient (1% B/min) at a flow-rate of 1 ml/min, where eluent A is 0.1% aq. TFA and eluent B is 0.1% TFA in acetonitrile. The ratio of the peaks is approximately 1:4:1 (S3:S4:S5). The main component (S4) represents the desired product. The smaller peaks represent more hydrophilic (S3) and hydrophobic (S5) peptide impurities. Multi-column SDC of this mixture provided the preparative data presented in Figs. 3–6. The sequences of synthetic peptides S3, S4 and S5 are shown in Experimental.

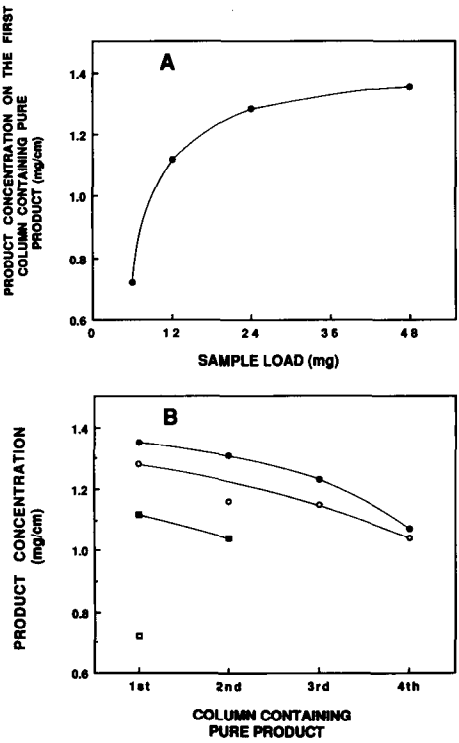

Fig. 3. Effect of sample load on distribution of peptide product, S4, from a three-decapeptide mixture (see Fig. 2) following multi-column SDC. These data are derived from results presented in Table II, with an additional sample load of 6 mg. (A) Relation between product (S4) concentration (mg/cm column) on the first column segment containing product only and the sample load; (B) relation between product (S4) concentration (mg/cm column) on each column segment containing product only and sample load. The first column to contain product only is nearest to the multi-column outlet; the fourth column is closest to the multi-column inlet. Symbols in (B): ● = 48; ○ = 24; ■ = 12; □ = 6 mg.

S4 was separated from S3 and S5 in four fractions at either flow-rate. The amount of pure S4 recovered in each of fractions 1–4 at 2 ml/min was less than that recovered from each of fractions 2–5 at 0.2 ml/min, *i.e.*, there was a dilution effect at the higher flow-rate, resulting in less product bound per individual column segment. The total pure S4 recovered at 2 ml/min was 8.6 mg; in contrast, at 0.2 ml/min, the total pure S4 recovered was 13.8 mg.

Effect of sample load. The 24-mg sample load was found to be optimal (Table II), with four fractions (2–5) containing pure S4 (a total of 13.8 mg). Although the 48-mg sample load also resulted in four pure fractions (1–4) of S4 (a total of 14.8 mg), about 50% of this peptide (16 mg) has been displaced into the breakthrough fraction, *i.e.*, the column was overloaded.

From Fig. 3A, the concentration (mg/cm of column) of peptide in the first column containing pure S4 increased with increasing sample load. From a rapid rise between sample loads of 6 mg and 24 mg, there was a levelling off of product concentration between 24 mg and 48 mg as the maximum column capacity was reached and exceeded.

From Fig. 3B, the concentration (mg/cm of column) of peptide in columns containing only S4 decreased with increasing column (fraction) number, *i.e.*, there was a general decreasing gradient of pure S4 in the direction of the column inlet (compare first and fourth column). Only one and two columns (out of six) contained pure S4 in the 6-mg and 12-mg sample loads, respectively; four out of six columns contained pure S4 in the 24-mg and 48-mg sample loads.

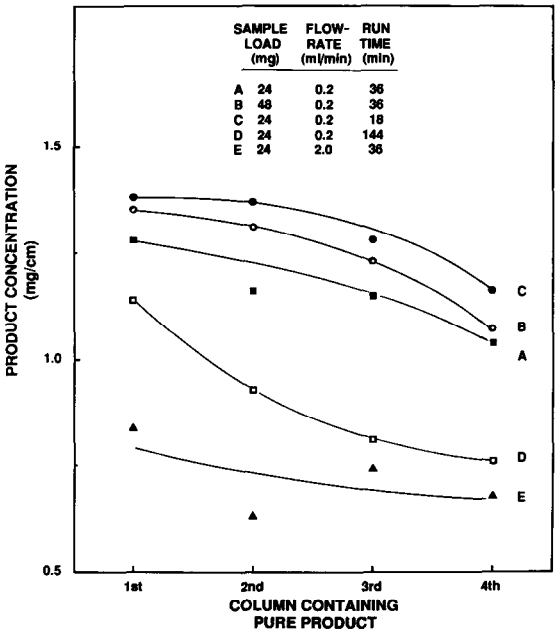

Fig. 4. Summary of effect of run parameters on distribution of pure peptide product, S4, following multi-column SDC of a three-decapeptide mixture (see Fig. 2). These data are derived from results presented in Table II and Fig. 3. The first column segment containing pure S4 is closest to the multi-column outlet; the fourth column is closest to the multi-column inlet.

Effect of run time. There was a general shift of peptide components down the total column length as run time increased (Table II). In addition, there was a dilution effect on the amount of S4 peptide recovered from each fraction containing pure S4 as run time increased.

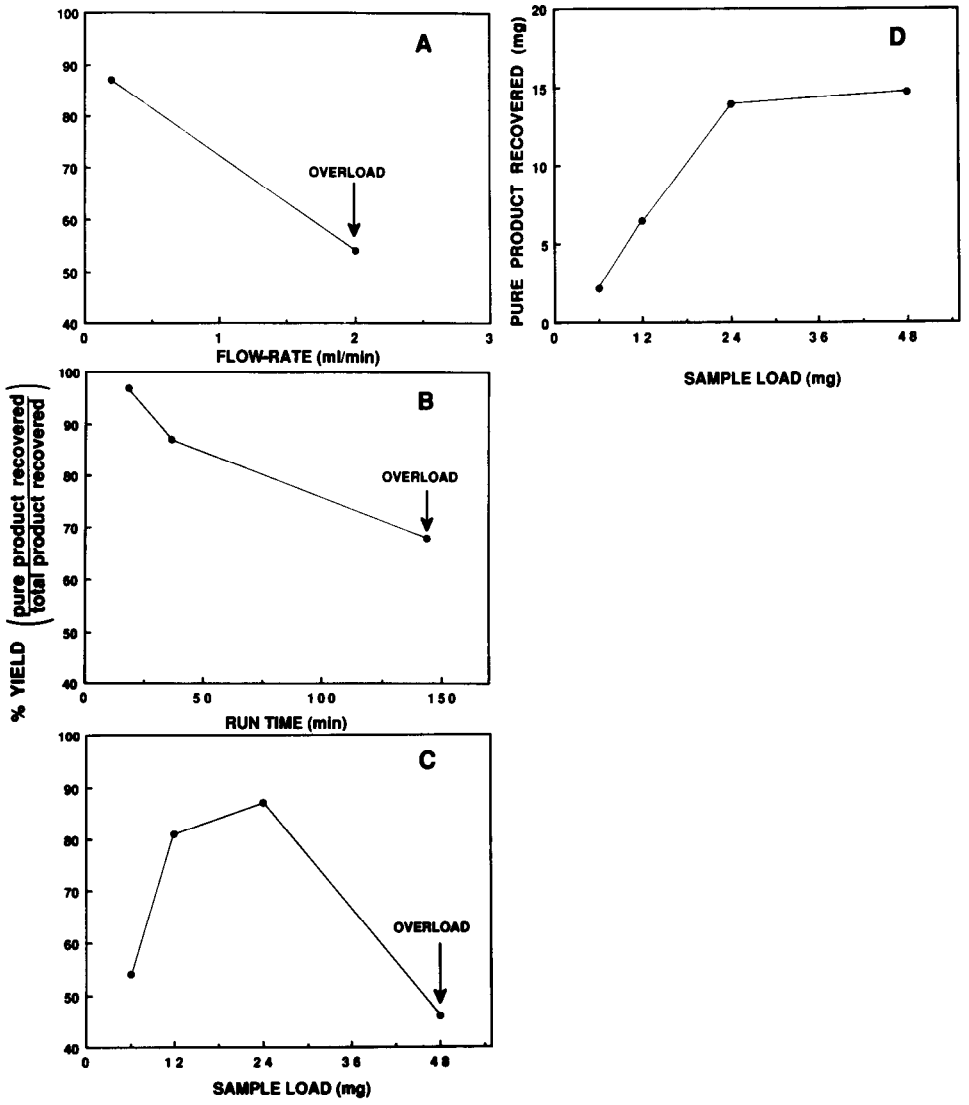

Fig. 5. Effect of varying run parameters on yield of pure peptide product, S4, following multi-column SDC of a three-decapeptide mixture (see Fig. 2). (A) Effect of flow-rate on yield of pure S4; (B) effect of run time on yield of pure S4; (C) effect of sample load on yield of pure S4. Overload conditions are defined as run conditions resulting in the desired peptide product being displaced from the column into the breakthrough fraction. (D) Amount of pure S4 recovered with increasing sample load.

TABLE II

EFFECT OF VARYING RUN PARAMETERS ON MULTI-COLUMN SDC OF A THREE-DECAPEPTIDE MIXTURE

Results are presented as amount of each peptide recovered (mg) from each fraction following SDC of the peptide mixture. An analytical profile of the mixture is shown in Fig. 2. Column, conditions and fraction analysis: see Table I. Effect of flow-rate: sample load and run time were 24 mg and 36 min, respectively. Effect of sample load: flow-rate and run time were 0.2 ml/min and 36 min, respectively. Effect of run time: flow-rate and sample load were 0.2 ml/min and 24 mg, respectively. The sequences of synthetic decapeptides S3, S4 and S5 are shown in Experimental.

Fraction number	Effect of flow-rate			Effect of load			Effect of run time		
	S3	S4	S5	S3	S4	S5	S3	S4	S5
	0.2 ml/min			*12 mg*			*18 min*		
0	2.3	0	0	0	0	0	0	0	0
1	1.7	1.8	0	0	0	0	4.0	0	0
2	0	3.8	0	0	0	0	0	4.1	0
3	0	3.5	0	2.0	0.3	0	0	4.1	0
4	0	3.4	0	0	3.4	0	0	3.8	0
5	0	3.1	0	0	3.1	0	0	3.5	0
6	0	0.3	4.0	0	1.2	2.0	0	0.5	4.0
	2 ml/min			*24 mg*			*36 min*		
0	4.0	6.2	0	2.3	0	0	2.3	0	0
1	0	2.5	0	1.7	1.8	0	1.7	1.8	0
2	0	1.9	0	0	3.8	0	0	3.8	0
3	0	2.2	0	0	3.5	0	0	3.5	0
4	0	2.0	0	0	3.4	0	0	3.4	0
5	0	1.2	1.0	0	3.1	0	0	3.1	0
6	0	0	3.0	0	0.3	4.0	0	0.3	4.0
				48 mg			*144 min*		
0		–		8.0	16.0	0	4.0	3.4	0
1		–		0	4.0	0	0	3.4	0
2		–		0	3.9	0	0	2.8	0
3		–		0	3.7	0	0	2.4	0
4		–		0	3.2	0	0	2.3	0
5		–		0	1.1	3.6	0	1.7	0.4
6		–		0	0	4.4	0	0	3.6

The shortest run time (18 min) was most efficient, with >90% of S4 loaded being recovered as pure peptide (a total of 15.5 mg from columns 2–5). The 144-min run time was the least efficient. The column was overloaded under these conditions with S4 in the breakthrough fraction (3.4 mg).

At each run time, four fractions contained S4 only. However, as shown in Table II, there was a general decrease in the amount of peptide recovered from each fraction containing pure S4 as run time increased (a total of 15.5 mg and 10.9 mg was recovered from the 18-min and 144-min runs, respectively).

Fig. 4 is a summary of the effect of varying SDC run parameters on distribution of pure peptide product, S4.

There was a general decrease in concentration (mg/cm column) of pure product with each successive column from the column outlet containing pure S4. Thus, comparing runs A and B, there was an increase in product concentration with increasing

sample load on each column containing pure S4; comparing runs A and C, there was an increase in product concentration with decreasing run time on each column containing pure S4; comparing runs A and D, there was a significant decrease in product concentration with a large increase in run time (four-fold) on each column containing pure S4; and, finally, comparing runs A and E, there was a significant decrease in product concentration with a large increase in flow-rate (ten-fold) on each column containing pure S4.

From Fig. 4, it is clear that SDC runs of low flow-rates and short run times at appropriate sample loads provided the highest concentration of pure product.

Effect of varying SDC run parameters on peptide yield. Fig. 5 shows the effect of various run parameters on yield of pure peptide product, S4, following multi-column SDC.

From Fig. 5A, it can be seen that there was a significant decrease in overall yield of pure S4 as the flow-rate was increased ten-fold (from 0.2 to 2 ml/min). This was due mainly to the fact that, at the higher flow-rate, SDC was being run under overload conditions (Table II).

From Fig. 5B, it is clear that there was a general decrease in yield of pure S4 as run time was increased. This effect is most apparent at the 144-min run time, where SDC capacity was exceeded (Table II), with S4 being displaced into the breakthrough fraction.

From Fig. 5C, it can be seen that there was an increase in yield of pure S4 as sample load was increased up to 24 mg, reflecting more efficient use of total column capacity with increasing peptide load. The sharp drop in yield of S4 at a sample load of 48 mg was due to the SDC capacity being exceeded (Table II), with S4 being displaced into the breakthrough fraction.

From Fig. 5D, it is apparent that there was an increase of pure S4 recovered with increasing sample load, up to 24 mg. At higher loads, there was no further increase in pure peptide recovered. From Table II, it can be seen that the 24-mg load was optimal in terms of both essentially saturating the individual columns and in the number of columns containing pure product. As the sample load was raised to 48 mg, all or most of the additional S4 loaded was lost to the breakthrough fraction (Table II). This result also reflected the flexibility of sample load during SDC. Thus, even under overload conditions, the same amount of pure product was recovered as in an ideal run, where sample load was just enough to reach total column capacity.

Based on the results presented in Figs. 3–5 and Table II, Fig. 6 represents the optimum SDC run for the three-decapeptide mixture (S3, S4 and S5), in terms of both efficient use of total column capacity and yield of purified S4.

In Fig. 6, the top elution profile shows the analytical preparation of the peptide mixture, carried out on the multi-column setup. Peptide S4 is the desired component. Following SDC, column 1 contained S3 only, while column 6 contained all of the S5 and a small amount of S4. Columns 2–5 contained the vast majority of the desired peptide component, S4, with no S3 or S5 present. It should be noted that, under these run conditions, all of the peptide sample was retained by the multi-column setup, *i.e.*, no peptide was found in the breakthrough peak.

Multi-column SDC of a synthetic peptide crude mixture

In order to examine the effectiveness of multi-column SDC in purifying a crude

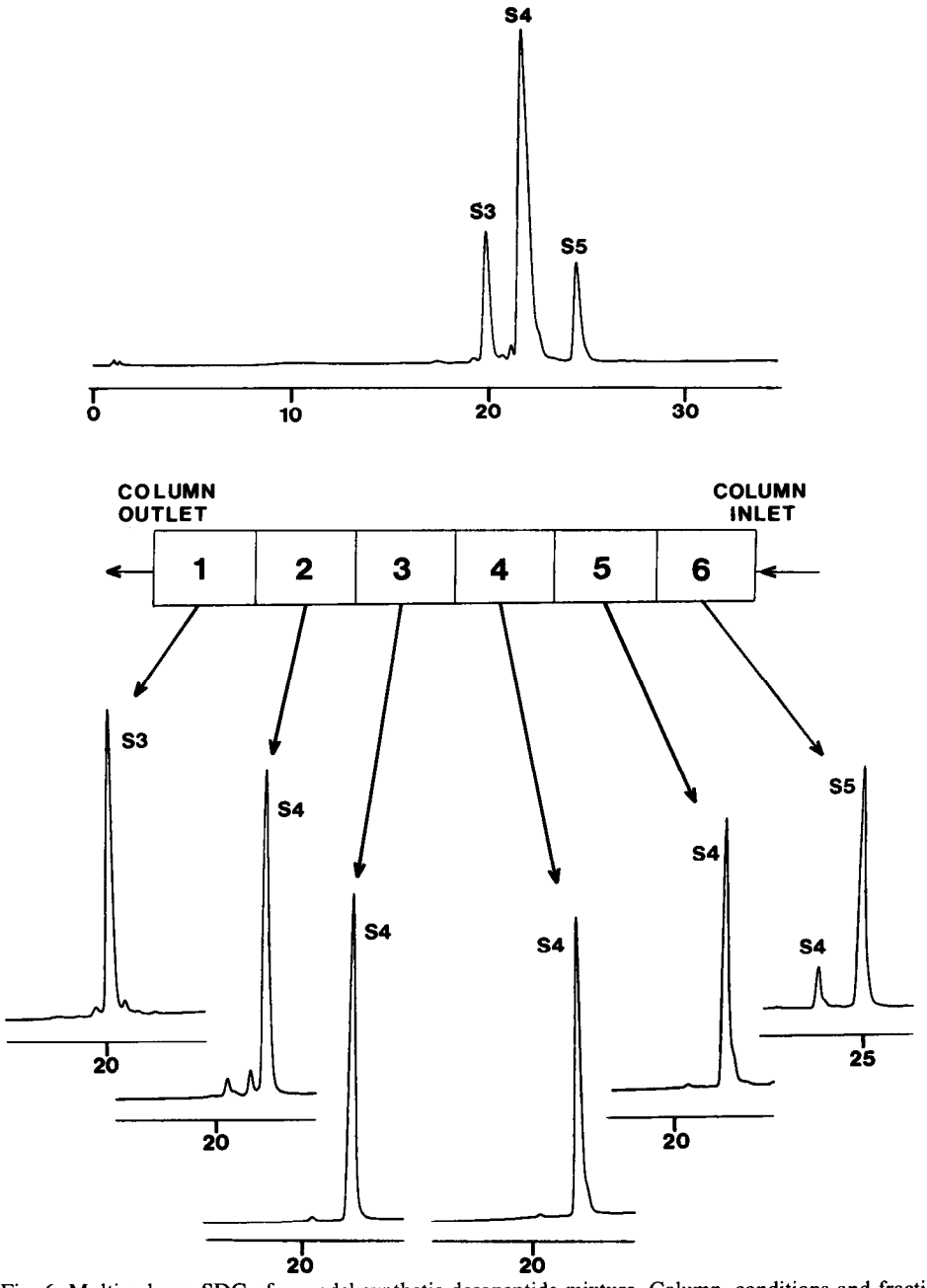

Fig. 6. Multi-column SDC of a model synthetic decapeptide mixture. Column, conditions and fraction analysis: see Table I. Flow-rate = 0.2 ml/min; run time = 18 min; sample load = 24 mg. The top elution profile shows the analytical separation of the mixture [linear AB gradient (1% B/min at 1 ml/min), where eluent A is 0.1% aq. TFA and eluent B is 0.1% TFA in acetonitrile]. The analytical elution profiles at the bottom show the peptide components retained on each individual column segment (columns 1–6) following the SDC run in 0.1% aq. TFA. The peaks are all correctly proportioned. The sequences of synthetic peptides S3, S4 (the desired product) and S5 are shown in Experimental.

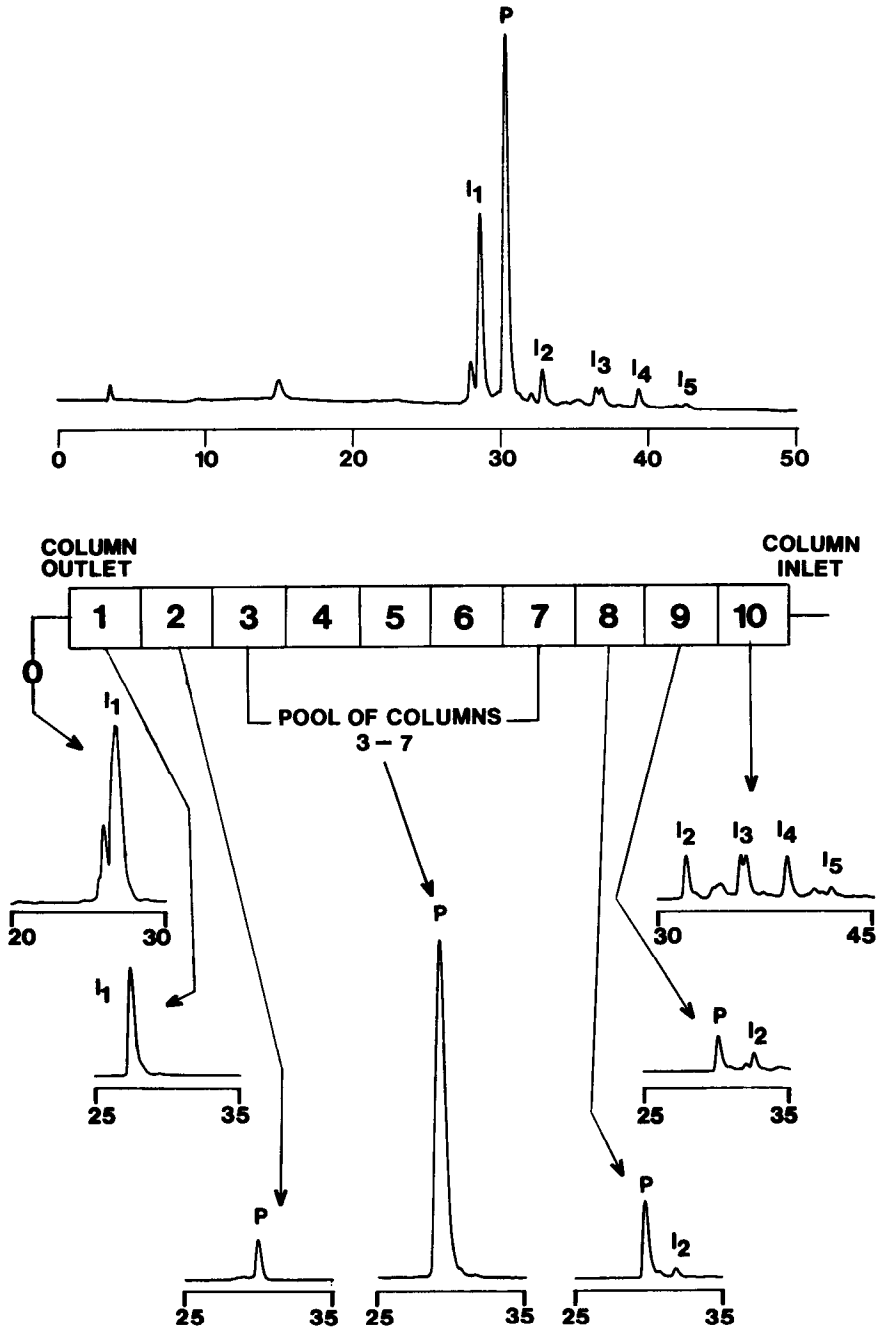

Fig. 7. Multi-column SDC of a synthetic decapeptide crude mixture. Column: ten 3 cm × 4.6 mm I.D. Aquapore RP300 C_8 column segments in series. Conditions and fraction analyses: as Fig. 6. Flow-rate = 0.5 ml/min; run time = 50 min; sample load = 100 mg. The top elution profile shows the analytical separation of the peptide mixture [linear AB gradient (1% B/min and 1 ml/min), where eluent A is 0.1% aq. TFA and eluent B is 0.1% TFA in acetonitrile]. The analytical elution profiles at the bottom show the peptide components retained on each individual column segment (columns 1–10) following the SDC run in 0.1% aq. TFA. The peaks are all correctly proportioned. *P* is the desired product; the other peaks are hydrophilic (I_1) and hydrophobic (I_2–I_5) impurities.

peptide mixture, as opposed to a model system, this technique was applied to the preparative separation of a synthetic decapeptide crude mixture.

In Fig. 7, 100 mg of a crude peptide sample has been applied to a multi-column setup, consisting of ten 3 cm × 4.6 mm I.D. Aquapore RP-300 C_8 column segments (columns 1–10) in series. The increase in the number of column segments (ten) compared to that employed previously (Figs. 3–6 and Table II) enabled the application of this substantial sample load.

The top elution profile shows the analytical separation of the crude synthetic peptide mixture. The large peak, *P*, is the desired peptide component; the smaller peaks are hydrophilic (I_1) and hydrophobic (I_2, I_3, I_4 and I_5) impurities.

Following SDC, the distribution of sample components through the ten column segments was determined. The breakthrough fraction (0) and column 1 contained only hydrophilic impurities (I_1). Column 10 contained only hydrophobic impurities (I_2–I_5) while columns 8 and 9 contained a small amount of peptide product contaminated with hydrophobic impurity I_2.

Columns 2–7 contained essentially pure product, the great majority of which was in columns 3–7. Of the total amount of desired peptide product loaded onto the column, 90% was recovered, with 81% of the peptide product isolated as pure peptide.

Very efficient use of the column capacity had been made during this SDC separation, which demonstrated well both the resolving power of this preparative process as well as the ease of fraction analysis.

CONCLUSION

In summary, this work describes an extension of a novel procedure for preparative reversed-phase separation of peptide mixtures, first reported by our laboratory [5–7]. Multi-column SDC enables the application of sample loads at least ten-fold greater than in comparable gradient elution experiments. Other advantages include minimal use of organic solvents and minimal fraction analyses. In addition, since the sample components act as their own displacers, no added displacer is required, unlike traditional displacement chromatography. Finally, rapid separations are achievable with multi-column SDC at a much lower cost (in terms of solvents, packings and machine use) than is typical for preparative gradient-elution separations. The potential of this technique is considerable (it is routinely used in the authors' laboratory) and should prove of great value to those involved in the purification of synthetic peptides.

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