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New polymer-based prepacked column for the reversed-phase liquid chromatographic separation of peptides over the pH range 2–12

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Abstract

The aim of this paper was to investigate the properties of a new column, Source™ 5RPC, for the separation of peptides at pH 2, 4.5, 7, 9 and 12 and to compare this product with similar polymer-based products available on the market. All columns were prepacked with 5 μm polystyrene–divinylbenzene polymer bead matrices and had dimensions of 150×4.6 mm I.D. Separations of angiotensin peptides were achieved on these columns using different equilibration solvents in the pH range 2–12 and elution with acetonitrile gradients. The separation of the peptide mixture obtained on Source 5RPC column was compared with that of two other commercially available polymer-based matrices. Method scouting and optimisation were carried out using the novel chromatography system, ÄKTA™ purifier.

Keywords: Source 5RPC columns; Stationary phases, LC; Peptides; Angiotensin

1. Introduction

Chromatographic separation of peptides is most commonly performed on silica-based matrices [1–6]. The disadvantage of using silica as a reversed-phase matrix is its instability at high pH; as a result, chromatography is restricted to a pH range of 2–8 [7,8].

Some basic peptides cannot be resolved in acidic conditions, resolution of these peptides is often achieved at high pH [8,9].

The excellent chemical stability of polymer-based matrices under basic conditions allows successful reversed-phase liquid chromatography (RPLC) at high pH [7–13]. Furthermore, these matrices withstand extreme cleaning procedures [7]. The advantages of RPLC at high pH include increased control of selectivity and improved solubility of basic samples [7,9].

In this paper we investigated and evaluated the separation of peptides on a new polymer-based column, Source 5RPC, over the pH range 2–12. The experiments were repeated on two other commercially available polymer-based columns for comparison with the Source 5RPC column.

2. Experimental[1]

2.1. Materials and columns

Angiotensin-I, angiotensin-III, Ile^{7}angiotensin-III and Val^{4}angiotensin-III were obtained from Sigma (St. Louis, MO, USA). Chromatography grade ace-

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[1]Source, ÄKTA and UNICORN are trademarks of Amersham Pharmacia Biotech Ltd. or its subsidiaries, 259VHP is a trademark of Separations Group, Milli-Q is a registered trademark of Millipore Corp. and Tris is a trademark owned by Rohn & Haas.

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Table 1
Amino acid sequences of the angiotensin peptides

Peptide	Sequence
Angiotensin-I	Asp–Arg–Val–Tyr–Ile–His–Pro–Phe–His–Leu
Angiotensin-III	Arg–Val–Tyr–Ile–His–Pro–Phe
Ile^{7}Angiotensin -III	Arg–Val–Tyr–Ile–His–Pro–Ile
Val^{4}Angiotensin-III	Arg–Val–Tyr–Val–His–Pro–Phe

tonitrile and trifluoroacetic acid (TFA) were from Merck (Darmstadt, Germany). All other chemicals used were of analytical grade.

The reversed-phase columns (all 150×4.6 mm I.D.) were: Source 5RPC ST column (code No. 17-5116-01) a product of Amersham Pharmacia Biotech (Uppsala, Sweden); PLRP-S 300 Å purchased from Polymer Labs. (Amherst, MA, USA) and Vydac 259VHP (Polymer RP) purchased from Vydac/Separations Group (Hesperia, CA, USA).

2.2. HPLC system

ÄKTA purifier 10 XT chromatography system, comprising of a model P-903 HPLC pump, a model UV-900 UV-monitor, a model pH/C-900 pH/con-

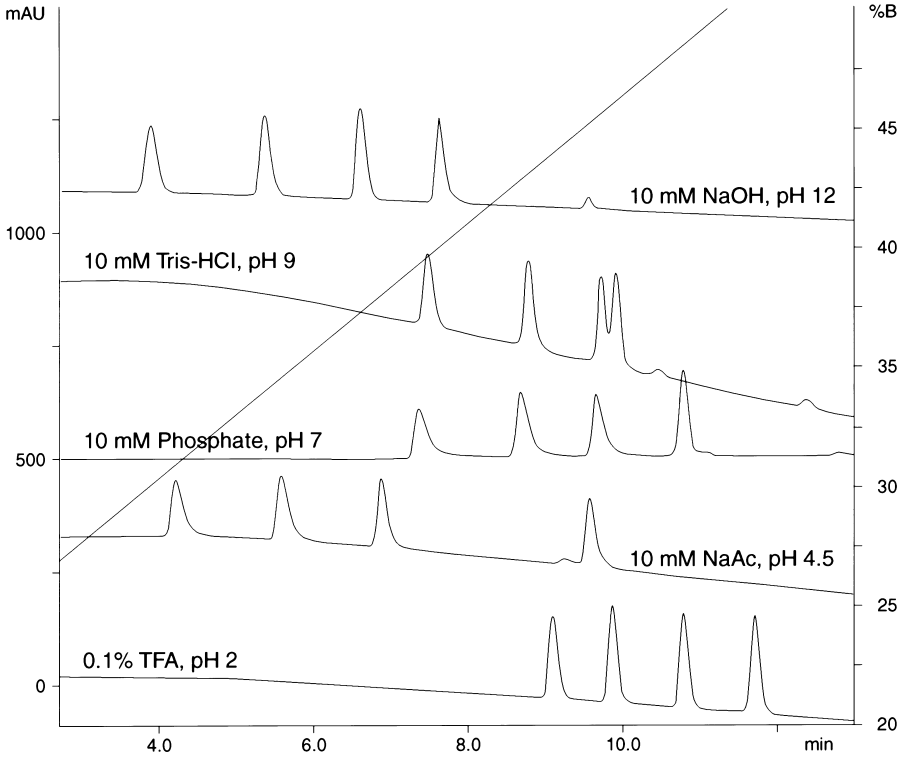

Fig. 1. Separation of Ile^{7}angiotensin-III, Val^{4}angiotensin-III, angiotensin-III and angiotensin-I by RPLC on a Source 5RPC ST 4.6/150 column at pH 2 (0.1% TFA), pH 4.5 (0.01 *M* sodium acetate buffer), pH 7 (0.01 *M* potassium phosphate buffer), pH 9 (0.01 *M* Tris HCl buffer) and pH 12 (0.01 *M* NaOH). Volume injected: 10 μl. Elution: linear gradient, 14–70% acetonitrile. Detection wavelength: 215 nm. For other details see Experimental. The figure is reproduced with permission from Amersham Pharmacia Biotech, 1998.

ductivity monitor, a model Frac-901 fraction collector, a model A-900 autosampler and UNICORN™ software system control were from Amersham Pharmacia Biotech.

2.3. Method

All the chromatography experiments were performed at room temperature at a flow-rate of 1 ml/min. The columns were equilibrated with five column volumes (CVs) of the equilibration solvents A1–A5, depending on the pH of the equilibration solvents (A1=0.1% TFA in Milli-Q water, pH 2; A2=0.01 *M* sodium acetate buffer, pH 4.5; A3=0.01 *M* potassium phosphate buffer, pH 7; A4=0.01 *M* Tris–HCl buffer, pH 9 and A5=0.01 *M* NaOH, pH 12). Ten microliters of a sample mixture containing 0.125 mg/ml of each peptide (see Table 1) prepared in Milli-Q water were injected into the columns. Elution was performed with solvent B (70% acetonitrile in Milli-Q water) using a 12-CV linear gradient from 20 to 100% B. The columns were cleaned after each experiment with three CVs 100% B solvent. The detection wavelength was 215 nm.

3. Results and discussion

Separation of angiotensin peptides was tested in the pH range 2–12 using a linear gradient elution (14 to 70% acetonitrile) on all the three columns described in Experimental. The elution order of the

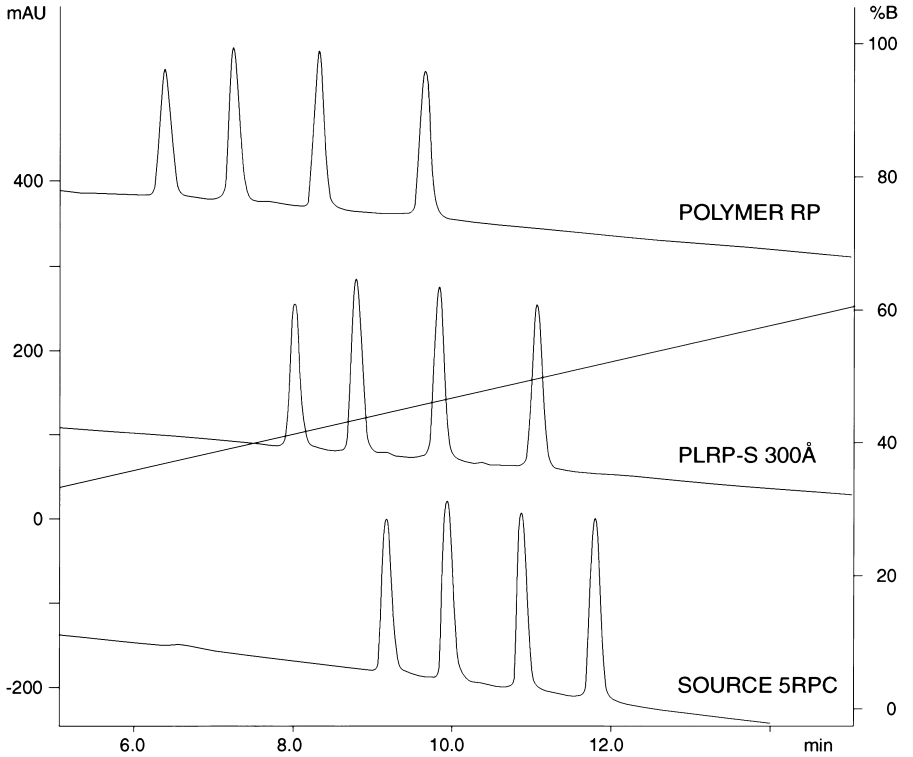

Fig. 2. Separation of Ile[7]angiotensin-III, Val[4]angiotensin-III, angiotensin-III and angiotensin-I by RPLC on Source 5RPC ST 4.6/150, PLRP-S 300 Å and Polymer RP columns at pH 2 (0.1% TFA). Volume injected: 10 μl. Elution: linear gradient, 14–70% acetonitrile. Detection wavelength: 215 nm. For other details see Experimental. The figure is reproduced with permission from Amersham Pharmacia Biotech, 1998.

peptides during all experiments was: Ile[7]angiotensin-III, Val[4]angiotensin-III, angiotensin-IJI and angiotensin-I as confirmed by individual runs of each peptide.

The results presented in Fig. 1 show chromatograms obtained from separations at pH 2, 4.5, 7, 9 and 12, using solvents A1 (0.1% TFA), A2 (0.01 *M* sodium acetate buffer), A3 (0.01 *M* potassium phosphate buffer), A4 (0.01 *M* Tris–HCl buffer) and A5 (0.01 *M* NaOH) respectively, on a Source 5RPC column. The peptide sample was resolved in four sharp peaks at each pH, but with differing selectivities. Thus, by altering the pH of the eluent, the selectivity of the column can be changed.

To compare different commercially available columns, similar experiments to those described above were repeated at pH 2, 7 and 12 on Source 5RPC ST 4.6/150, PLRP-S 300 Å and Polymer RP columns. These experiments were performed using the following solvents: A1 (0.1% TFA), A3 (0.01 *M* potassium phosphate buffer), and A5 (0.01 *M* NaOH). The results are presented in Figs. 2–4.

It can be seen in Fig. 2 that the peptide mixture was resolved as four sharp peaks on all three columns with some differences in retention times, indicating that the Source 5RPC column is more hydrophobic than the other two columns.

Fig. 3 shows the chromatograms resulting from separations of the angiotensin peptides performed on the described columns at pH 7. These chromatograms show that the best separation and sharpest peaks were achieved with the Source 5RPC column. This indicates that the Source 5RPC column has lower non-specific interactions with the peptides than the other two columns at neutral pH.

Comparing the different columns at high pH, Source 5RPC gave somewhat sharper peaks and higher retention times than the other two columns (see Fig. 4). The higher retention times indicate that

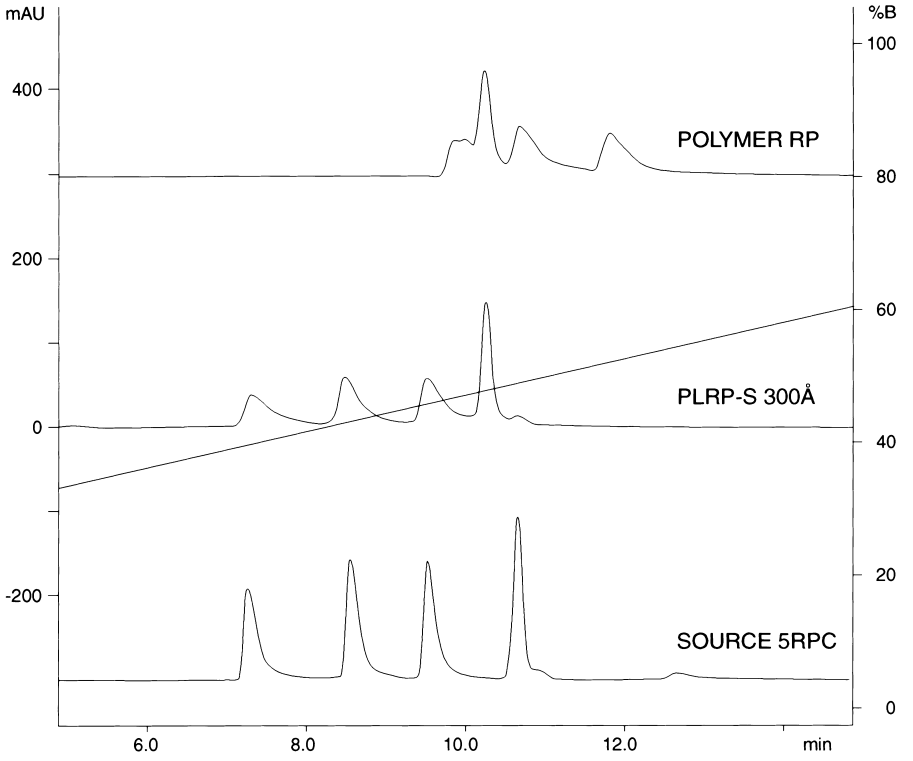

Fig. 3. Separation of Ile[7]angiotensin-III, Val[4]angiotensin-III, angiotensin-III and angiotensin-I by RPLC on Source 5RPC ST 4.6/150, PLRP-S 300 Å and Polymer RP columns at pH 7 (0.01 *M* potassium phosphate buffer). Volume injected: 10 μl. Elution: linear gradient, 14–70% acetonitrile. Detection wavelength: 215 nm. For other details see Experimental. The figure is reproduced with permission from Amersham Pharmacia Biotech, 1998.

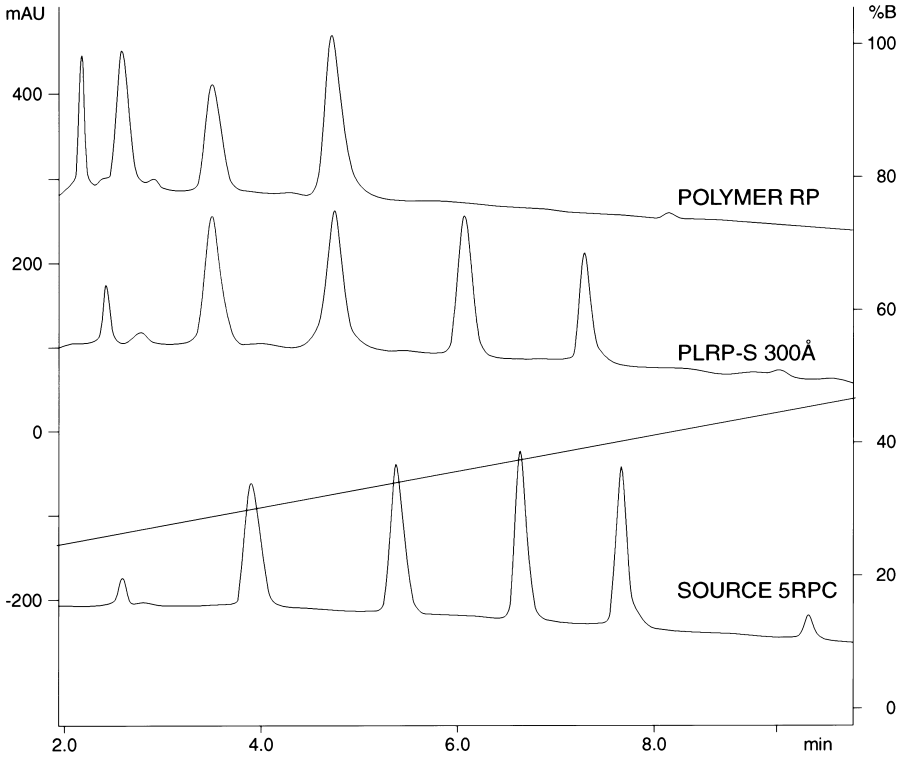

Fig. 4. Separation of Ile[7]angiotensin-III, Val[4]angiotensin-III, angiotensin-III and angiotensin-I by RPLC on Source 5RPC ST 4.6/150, PLRP-S 300 Å and Polymer RP columns at pH 12 (0.01 *M* NaOH). Volume injected: 10 μl. Elution: linear gradient, 14–70% acetonitrile. Detection wavelength: 215 nm. For other details see Experimental. The figure is reproduced with permission from Amersham Pharmacia Biotech (Uppsala, Sweden) 1998.

Source 5RPC is more hydrophobic than the other two columns.

4. Conclusions

1. Source 5RPC is a suitable RPLC matrix for the separation of peptides over the pH range 2–12.
2. Source 5RPC gives low non-specific interactions with the peptide sample in the pH range 2–12, compared with the other commercially available polymer columns tested.
3. Source 5RPC is more hydrophobic than the other commercially available polymer-based RPLC matrices tested.
4. Separation of peptides on Source 5RPC column is better than that achieved on other commercially available polymer-based RPLC columns, especially at neutral pH.

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