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Poster #140

Fast Peptide Separations at Elevated Temperatures by Microbore HPLC

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ABSTRACT

Conventional gradient HPLC peptide separations are performed on silica-based reversed phase columns having alkyl bonded phases. Peptides are retained by the hydrophobic packing material in the presence of aqueous trifluoroacetic acid (TFA) and eluted with a gradient of increasing organic solvent, usually acetonitrile. The separation is normally performed at ambient or slightly elevated temperature (~35°C). Peptide separations can be optimized by adjustments in flow rate, column diameter and length, particle size, gradient slope and temperature. Separations with shallow gradients on long, narrow columns packed with small particles provide maximum resolution, but may require reduced flow rates and extended run times. Separations on large-bore columns packed with large particles can provide rapid separations through increased flow rates, but often result in reduced resolution. When sample amount is limiting, narrow bore (i.d.= 2 mm) and microbore (i.d.=1 mm) columns packed with small particles provide increased sensitivity since peptides are eluted in a higher concentration. At ambient temperature, separations on narrow bore columns are performed at reduced flow rates (<1 ml/min). Operating at elevated temperatures will decrease solvent viscosity and increase the fluidity of the alkyl bonded phase (C18). This can result in altered selectivity and decreased run times. Such alterations can lead to optimized high-speed peptide separations on narrow bore columns.

MATERIALS AND METHODS

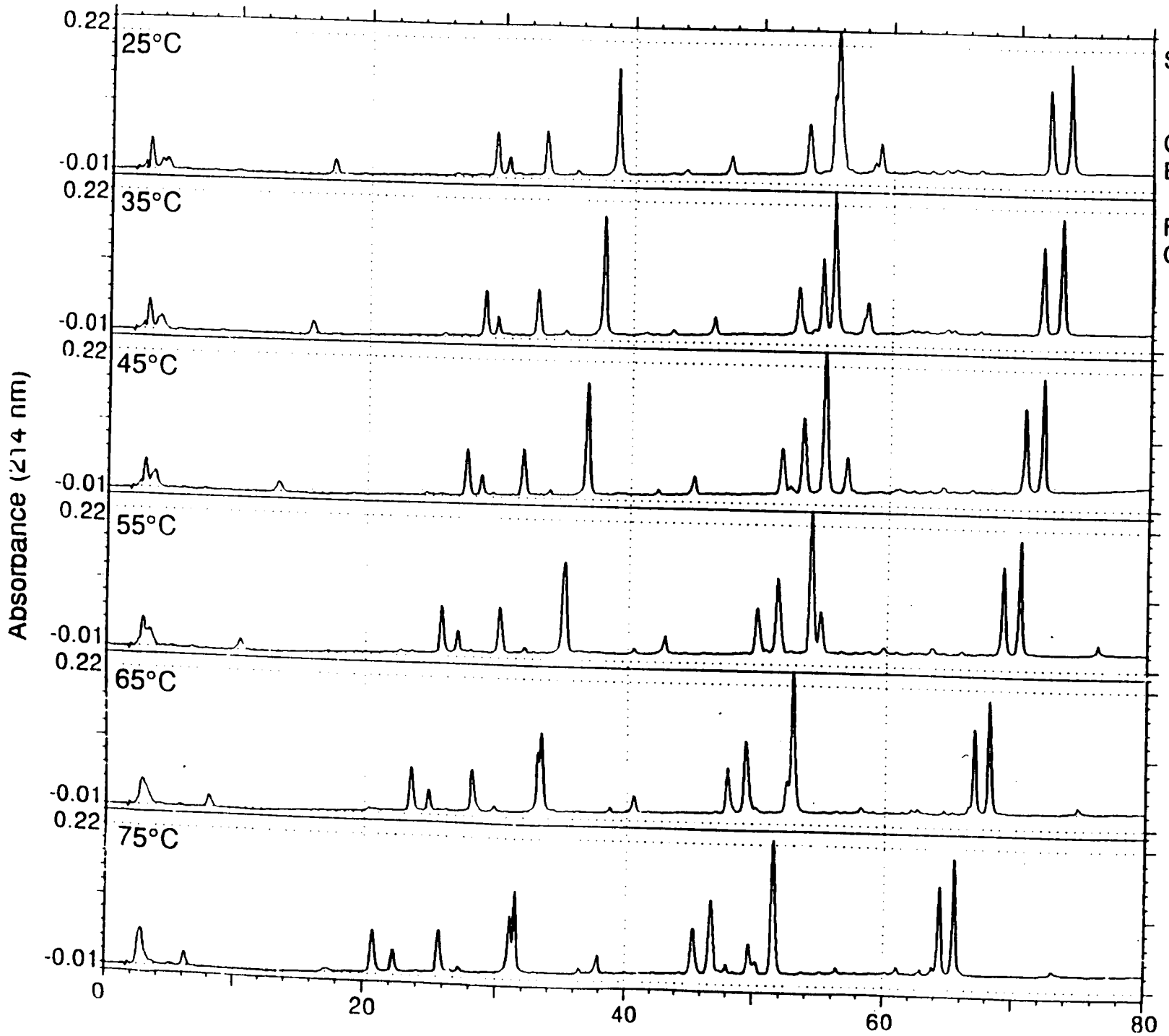
Tryptic Digestion

Cytochrome *c* (Sigma Chemical Co) from bovine heart mitochondria (1mg/500µl) were suspended in 0.1M ammonium bicarbonate (Sigma) buffer, pH 8.0. TPCK-treated Trypsin (Worthington Biochemical Corp.) was dissolved at a concentration of 0.2mg/1ml buffer. Trypsin solution (500µl) was added to the cytochrome *c* suspensions and incubated for 24h at 37°C. Following incubation, trypsin was deactivated by heating at 100°C for 5 min. Aliquots of 100 µl were frozen (-20°C). Prior to HPLC, digests were diluted 1:10 with aqueous TFA.

HPLC System

Samples were chromatographed on a 625 LC System (Waters Division of Millipore) equipped with a column heater and autosampler (Waters model 715). Samples were analyzed by photodiode array detection (Waters model 991) in a wavelength range of 190-425 nm with 1.3 nm resolution. Peptide mixtures were separated on Waters columns (2 mm x 150 mm) packed with C4, C8, or C18 Delta-Pak™ reversed phase supports. All particles were 5µm with 300Å pore sizes.

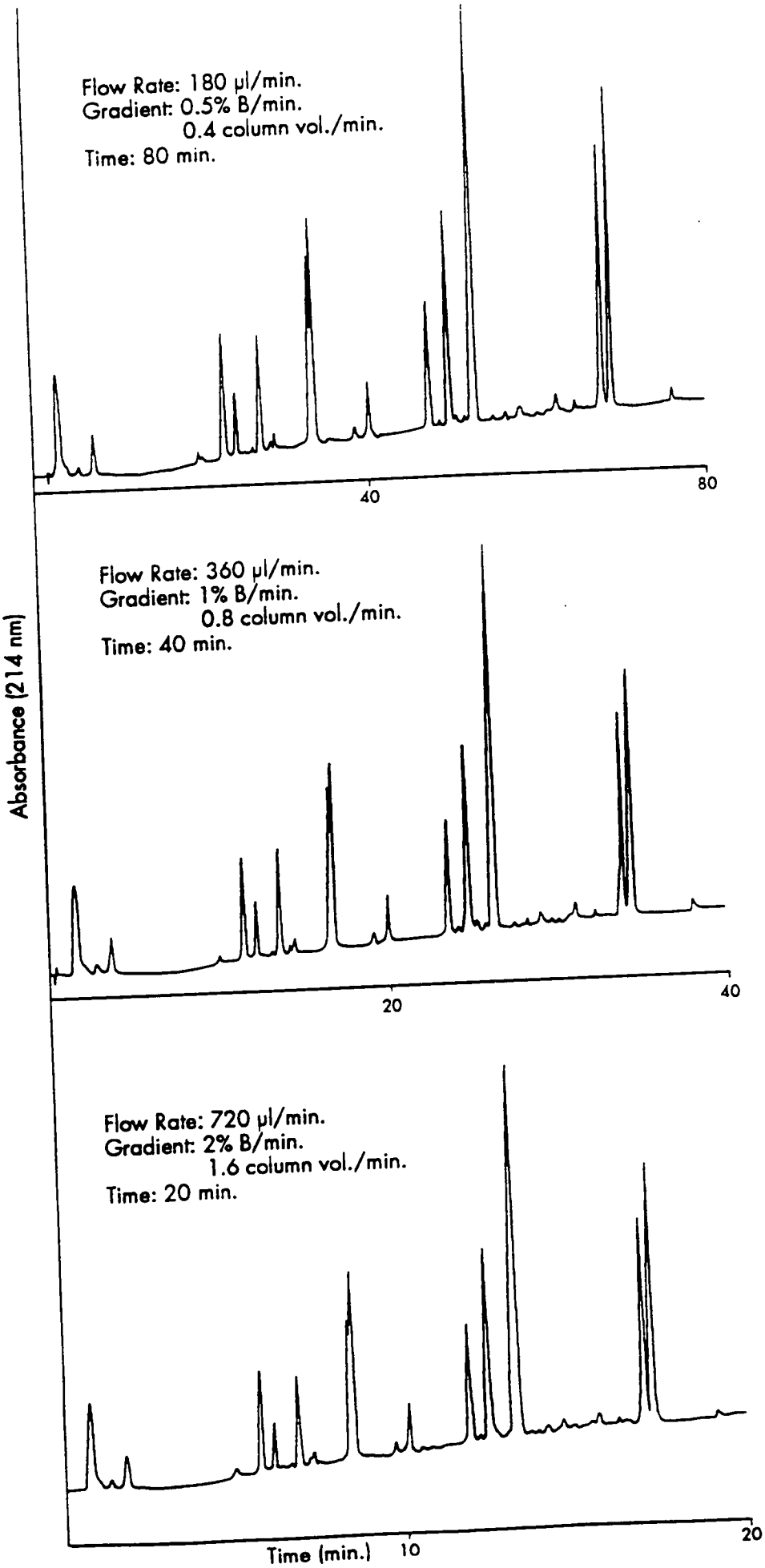
Effect of Temperature on Peptide Mapping: C4 Reversed Phase Support



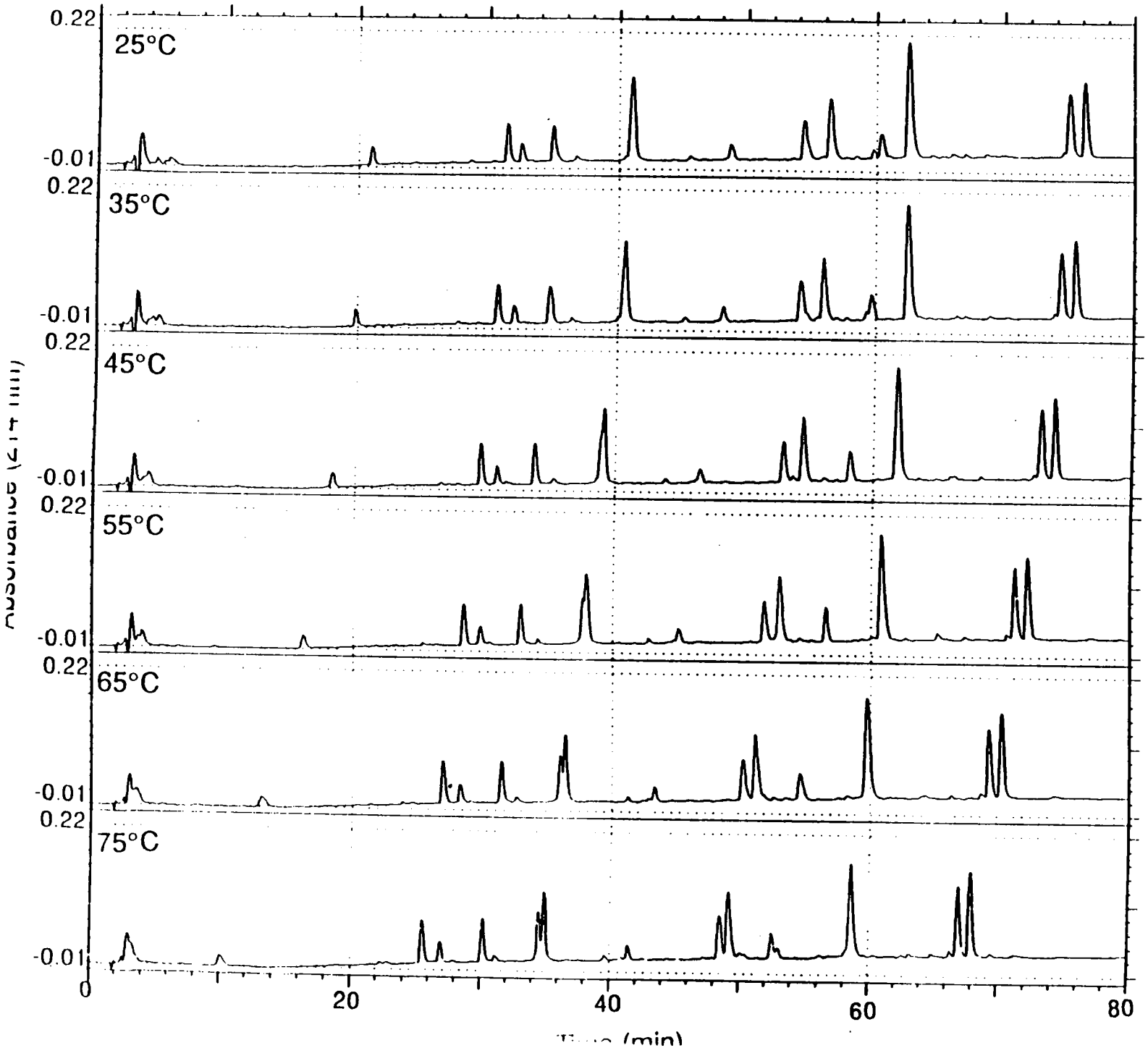
Sample: Tryptic Digest
Bovine Cytochrome *c*
(400 pmol)
Column: C4 (2mm x 150 mm)
Eluants: A=water/0/1% TFA
B=acetonitrile/0.1% TF
Flow: 180 µl/min
Gradient: 0-60% B/120 min
(46 column volumes)

High-Speed Separation on Narrow-Bore C_4 Column at 75°C

Eluants
A = Water/0.1% TFA
B = Acetonitrile/0.1% TFA



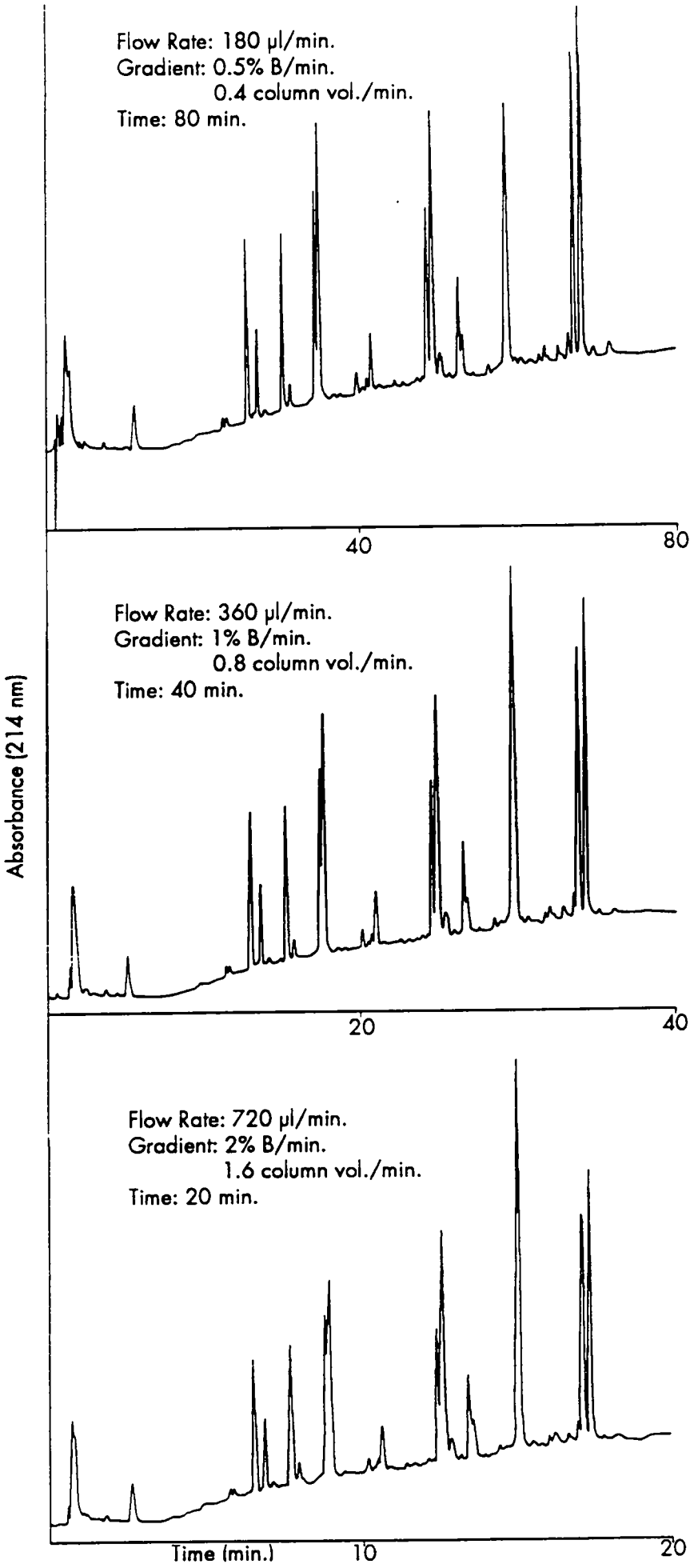
Effect of Temperature on Peptide Mapping: C18 Reversed Phase Support



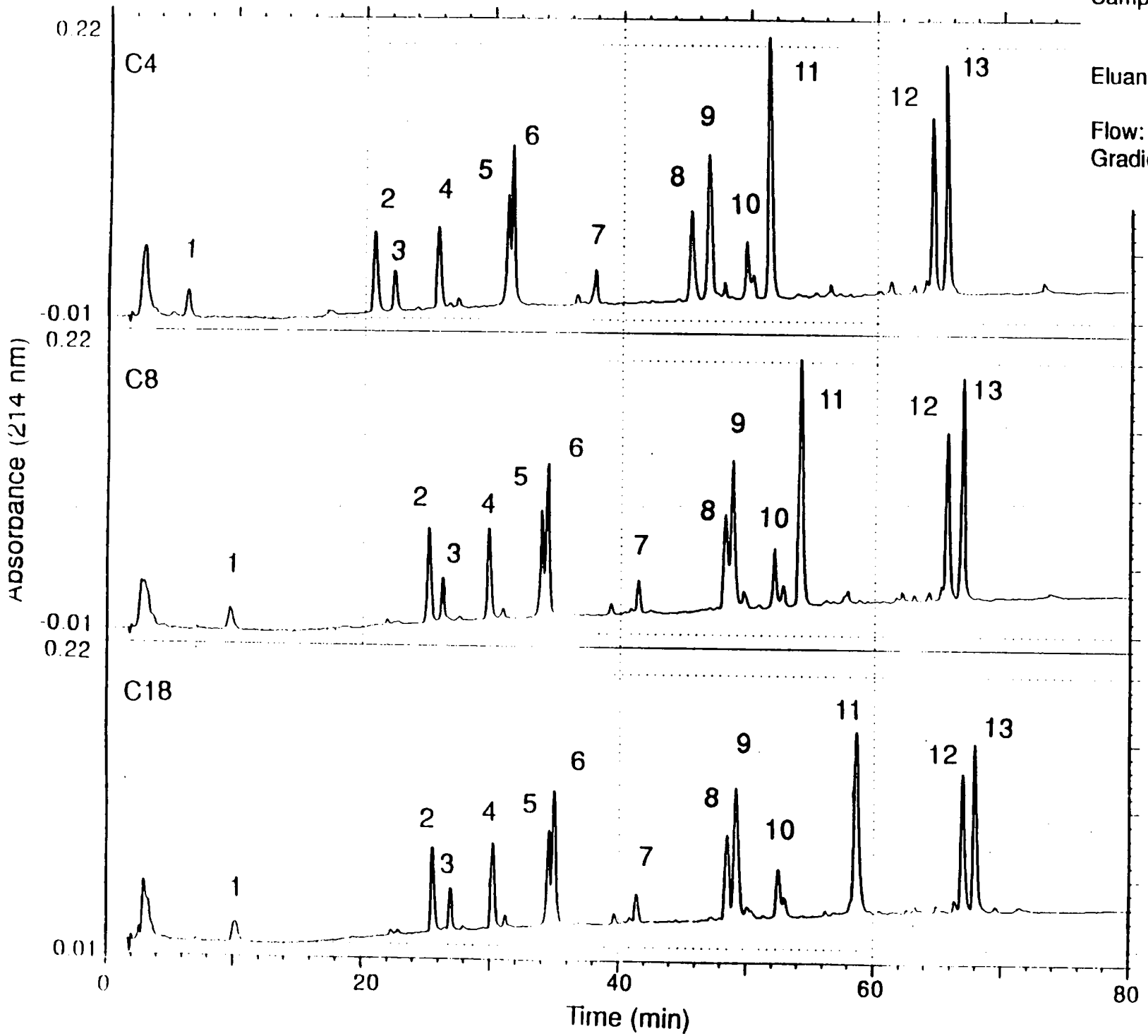
Sample: Tryptic Digest
Bovine Cytochrome *c*
(400 pmol)
Column: C18 (2mm x 150 mm)
Eluants: A=water/0/1% TFA
B=acetonitrile/0.1% TFA
Flow: 180 µl/min
Gradient: 0-60% B/120 min
(46 column volumes)

High-Speed Separation on Narrow-Bore C_{18} Column at 75° C

Eluants
A = Water/0.1% TFA
B = Acetonitrile/0.1% TFA



Selectivity Differences Between Reversed Phase Supports at 75°C



Sample: Tryptic Digest
Bovine Cytochrome *c*
(400 pmol)

Eluants: A=water/0/1% TFA
B=acetonitrile/0.1% TFA

Flow: 180 µl/min

Gradient: 0-60% B/120 min
(46 column volumes)

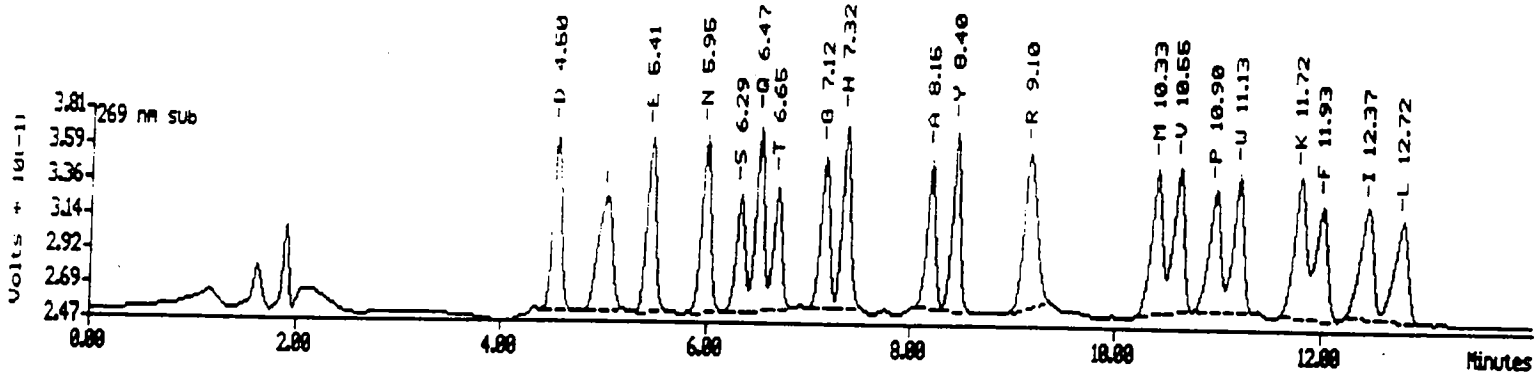
Amino acid sequence for tryptic peptides derived from bovine cytochrome c.

Peptide number		Bovine Sequence
1	(1-5)	GDVEK
2	(74-79)	YIPGTK
3	(73-79)	KYIPGTK
4	(9-13)	IFYQK
5	(39-53)	KTGQAPGFSYTDANK
6	(40-53)	TGQAPGFSYTDANK
7	(92-97)	EDLIAY
8	(80-86)	MIFAGIK
9	(28-38)	TGPNLHGLFGR
10	(92-99)	EDLIAYLK
11	(14-22)	CAQCHTVEK (+Heme)
12	(56-73)	GITWGEETLMEYLENPKK
13	(56-72)	GITWGEETLMEYLENPK

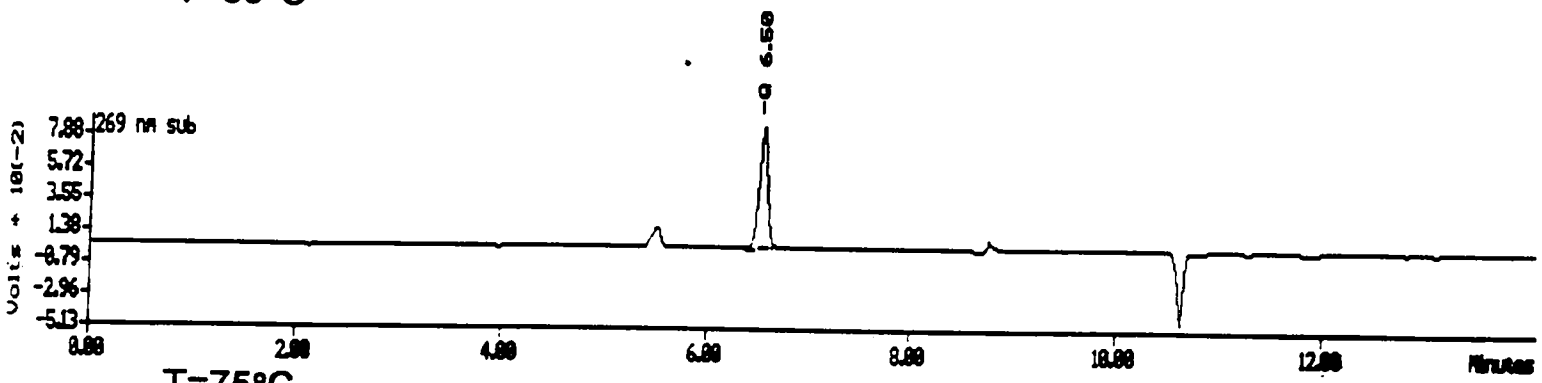
Results from Sequencing Peptide 4 (IFYQK)

It is possible that exposure to high temperature (75°C) may cause deamination of glutamine (Q) to glutamic acid (E). To test the effect of high temperature on the recovery of glutamine, peptide 4 was isolated from separations run at 35°C and 75°C and analyzed on a ProSequencer™ (Millipore model 6625) automated peptide sequencer.

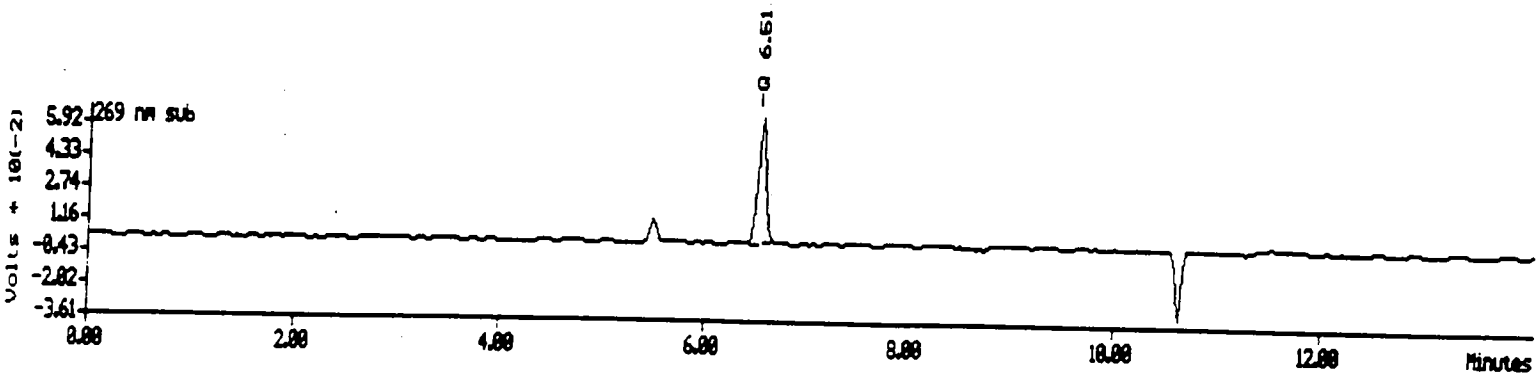
PTH Standard



T=35°C



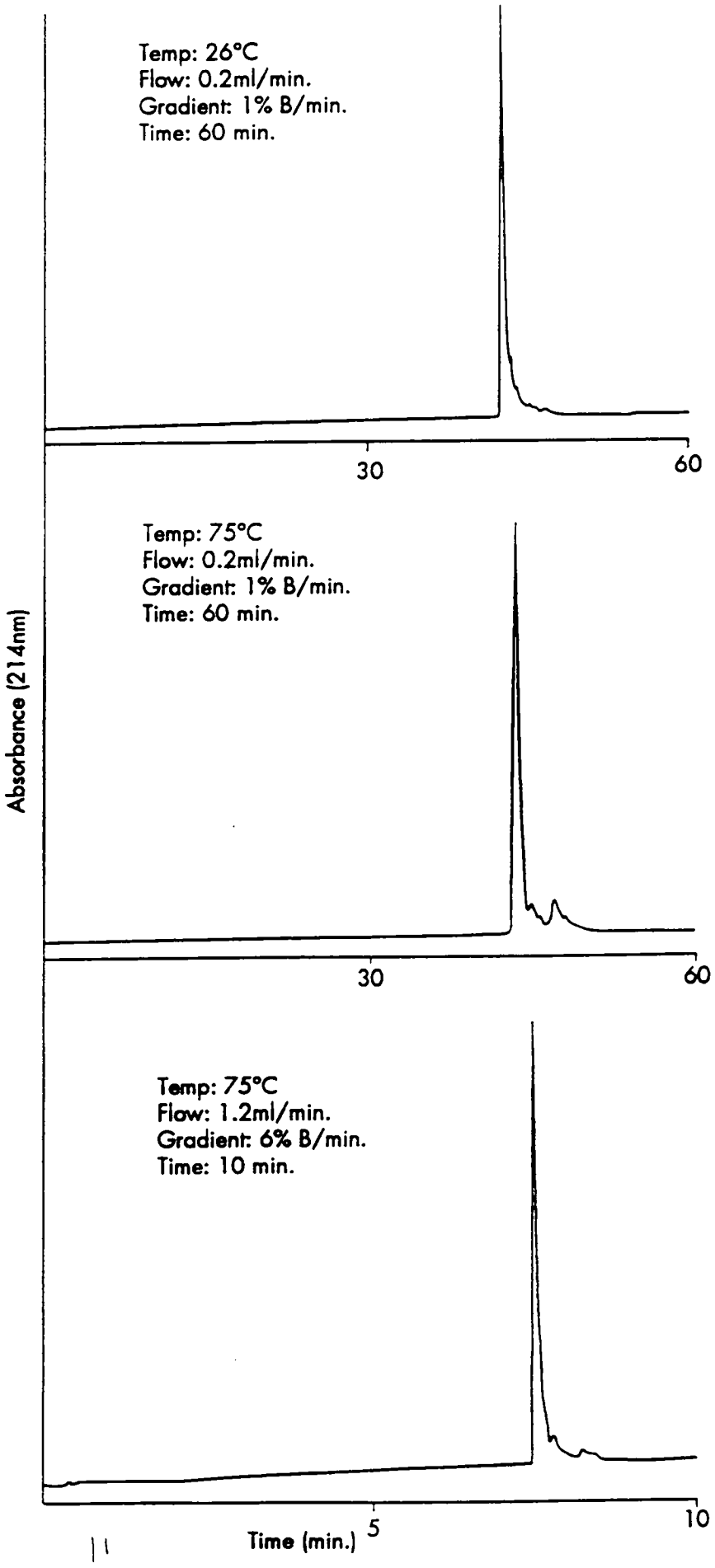
T=75°C



These data indicate that isolation of peptides having amide-based residues are not adversely affected by purification at 75°C.

Effect of Temperature and Gradient Slope: Separation of Insulin on Narrow Bore C_{18} Column

Eluants
A = Water/0.1% TFA
B = Acetonitrile/0.1% TFA



CONCLUSIONS

- Fast peptide separations can be performed at elevated flow rates and temperatures on silica-based reversed phase supports.

- High-speed HPLC methodologies can be used to prepare peptides for sequencing and amino acid analysis. These techniques can be useful to rapidly monitor the progress of proteolytic digestions, preparative processes, chemical modifications, and other on-going protocols.

- For columns operated at the same flow rate, increasing the operating temperature enhanced resolution. At 75°C, increasing the flow rate and gradient slope resulted in equivalent chromatograms in less time.

- For each chain length studied (C_4,C_8, or C_{18}), increasing column temperature resulted in increased resolution and altered selectivity.

930656

139 Use of Short Microcolumn for Fast Analysis of Proteins and Peptides. RICHARD LUDWIG, Supelco, Inc., Supelco Park, Bellefonte, PA 16823-0048 USA.

The mass sensitivity and low solvent consumption of small diameter columns have been clearly demonstrated (1). The use of short 1.0mm diameter columns packed with wide pore materials for the rapid analysis of protein and peptide microsamples will be demonstrated. These columns give high sensitivity/recovery with limited sample sizes. In addition the low flow rates employed facilitate coupling with mass spectrometers. When coupled to MS/MS systems both molecular weight and sequence information can be obtained for peptides (2).

(1)Small Bore Liquid Chromatography Columns: Their Properties and Uses, Vol. 72 Chemical Analysis Series, R.P.W. Scott (Ed.) John Wiley & Sons, New York (1984).
(2)E.C. Huang, T. Wachs, J.J. Conboy, and J.D. Henion, Anal. Chem., 62, 713A (1990).

* **140 Fast Peptide Separations at Elevated Temperatures by Microbore HPLC.** PATRICIA YOUNG*, HAROLD RICHARDSON, THOMAS WHEAT, and GEORGE VELLA, Millipore Corporation, 34 Maple St., Milford, MA 01757 USA.

Conventional gradient HPLC peptide separations are performed on silica-based reversed phase columns having alkyl bonded phases. Peptides are retained by the hydrophobic packing material in the presence of aqueous trifluoroacetic acid (TFA) and eluted with a gradient of increasing organic solvent, usually acetonitrile. The separation is normally performed at ambient or slightly elevated temperature (~35°C). Peptide separations can be optimized by adjustments in flow rate, column diameter and length, particle size, gradient slope and temperature. Separations with shallow gradients on long, narrow columns packed with small particles provide maximum resolution, but may require reduced flow rates and extended run times. Separations on large-bore columns packed with large particles can provide rapid separations through increased flow rates, but often result in reduced resolution. When sample amount is limiting, narrow bore (i.d. = 2 mm) and microbore (i.d. = 1 mm) columns packed with small particles provide increased sensitivity since peptides are eluted in a higher concentration. At ambient temperature, separations on narrow bore columns are performed at reduced flow rates (< 1 ml/min). Operating at elevated temperatures decreases solvent viscosity and increases the fluidity of the alkyl bonded phase (C18). This can result in altered selectivity and decreased run times. Such alterations can lead to optimized high-speed peptide separations on narrow bore columns.

141 Analysis and Process Control of Polymers by HPLC and GPC. STEPHAN ROSE*, MARIO MELZI, NICO VONK and WILL VERSTRAETEN, Chrompack Int. B.V., P.O. Box 8033,4330 EA Middelburg, THE NETHERLANDS.

This paper describes two methods for polymer analysis which can be used for process control as well as for quality control: 1. Low temperature PET analysis. 2. Analysis of isocyanate polymers.

Low temperature PET analysis. Polyethylene Terephtalates (PET) are used more and more as packaging material for food and beverages. For this reason the product is subjected to various residual monomer and degradation studies The GPC method presented allows molecular weight (MW) distribution analyses of PET at ambient temperatures and can be used as a MW- screening technique. Data analysis of the low MW fraction gives information about residual monomers, additives and degradation products. Analysis of the overall MW distribution allows monitoring of polymer degradation.

Thus the method described gives the complete information of both monomer content as well as molecular size distribution in one single analysis.

Analysis of isocyanate polymers. The analysis of isocyanates is not only important for process control but also for environmental analysis, because of the toxicological problems related to the monomers and oligomers.

The polymerization process of isocyanates like MDI and TDI can be monitored by low molecular weight GPC, as well as by RPLC. In this paper we compare both techniques. The RPLC method uses a specially modified ODS column and can be used for rapid process control of oligomeric samples as well as for environmental monitoring. Both methods are compared on speed, resolution, MW data and application range.