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THE USE OF ON-LINE SAMPLE CONCENTRATION TO INCREASE THE SENSITIVITY OF CAPILLARY ELECTROPHORESIS

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THE USE OF ON-LINE SAMPLE CONCENTRATION TO INCREASE THE SENSITIVITY OF CAPILLARY ELECTROPHORESIS.

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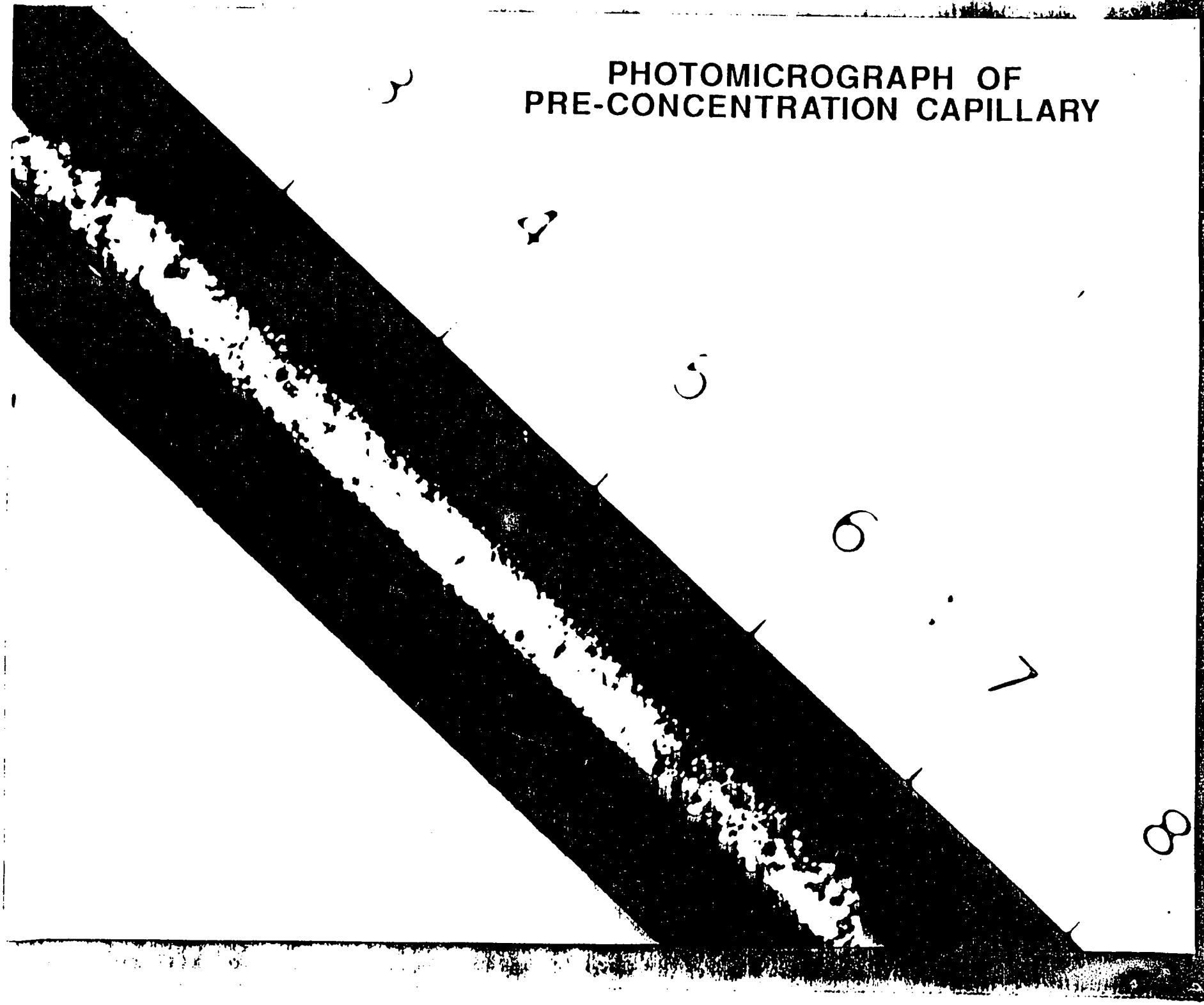
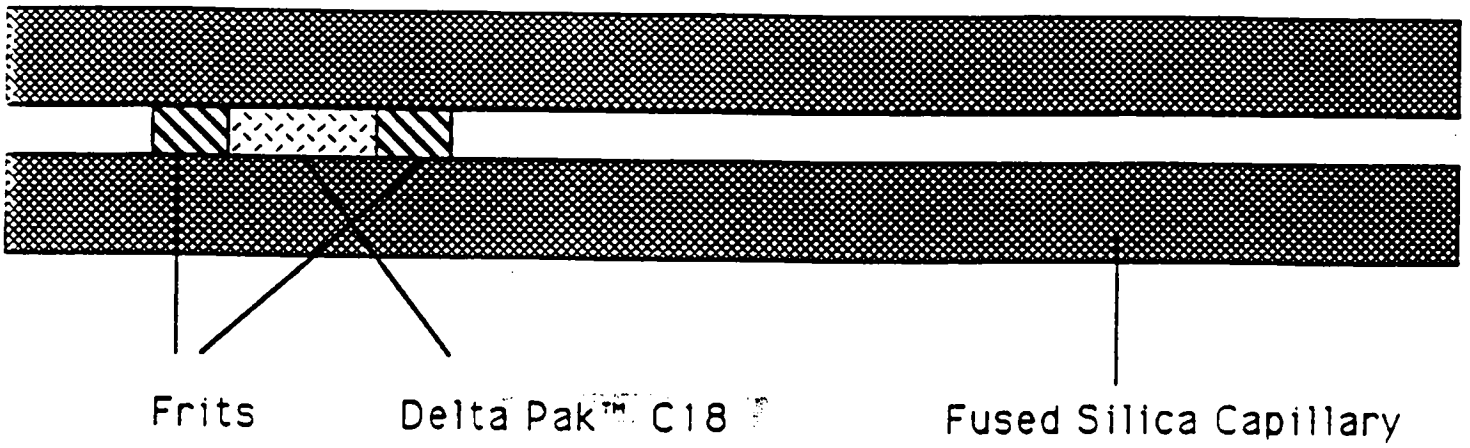
Capillary Electrophoresis is a high resolution separation technique that is of growing importance in the bioanalytical laboratory. Using on-line UV detection, as little as 10pg of a peptide may be detected. However, because injection volumes are limited to 1-10nl, the lower limit for detection with respect to sample concentration is about 1ug/ml. In order to lower this limit we have constructed a fused silica capillary that contains a packed bed of C18 chromatographic material with a length of 1-2mm at the injection end. Large volumes of dilute peptide samples were injected using conditions where the sample binds to the C18. After sample loading, a small volume of organic solvent was injected and electrophoresis conducted in the normal fashion. Using this technique, peptides have been detected at original sample concentrations down to 1ng/ml.

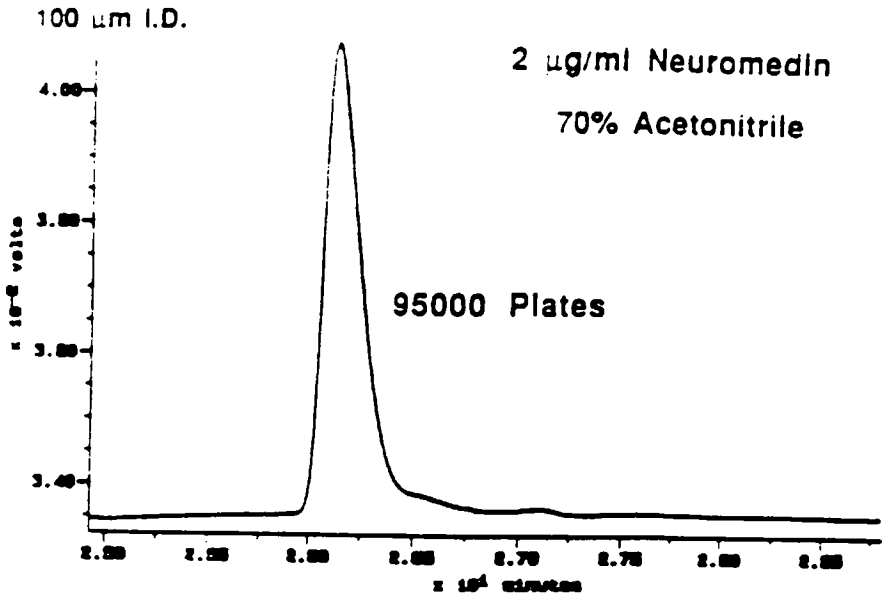
PHOTOMICROGRAPH OF PRE-CONCENTRATION CAPILLARY

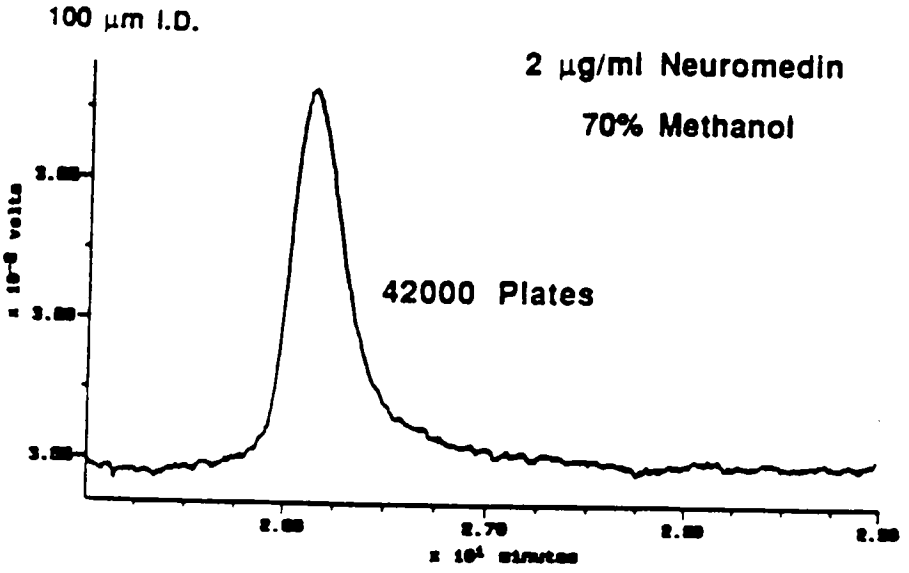


Pre-Concentration Capillary



EFFECT OF ORGANIC SOLVENT ON PLATE COUNT





Conditions:

Capillary:	100 µm x 60 cm, 1.5 mm Delta-Pak™
Buffer:	25 mM Na Citrate, pH 3.5
Eluent:	25 mM Na Citrate, pH 3.5/Indicated Organic 25/75
Sample:	2 µg/ml Neuromedin in Buffer
Injection:	500 seconds, 8 kV
Elution:	20 seconds, 7 kV
Run:	8 kV

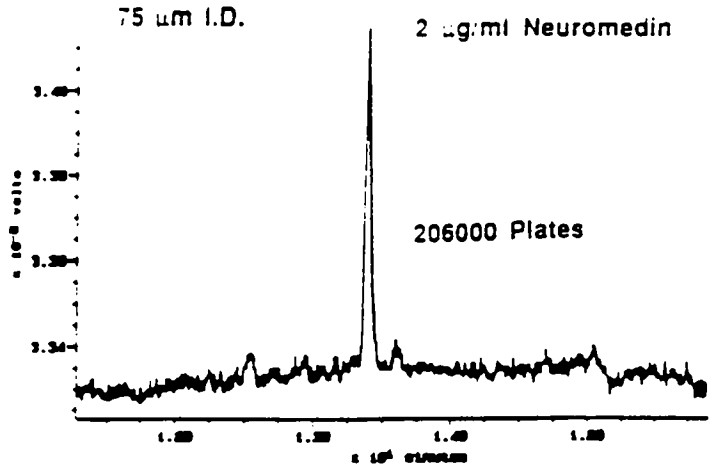
Reproducibility Using A Preconcentration Capillary

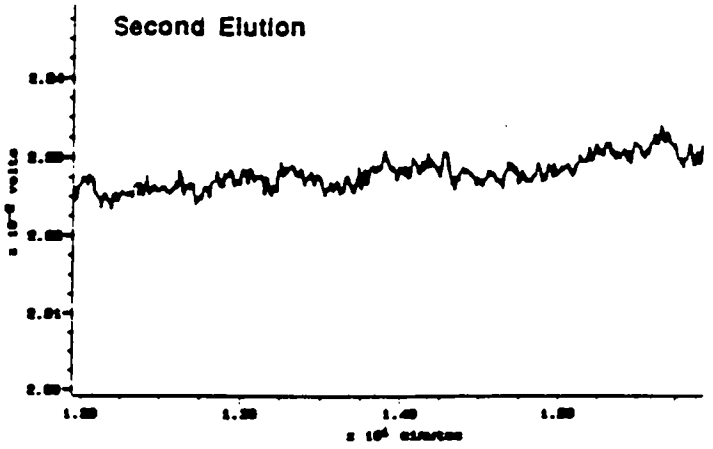
Experimental:

Capillary:	100 µm x 60 cm , 1.5 mm Delta-Pak
Buffer:	25 mM Sodium Citrate, pH 3.5
Eluent:	10 mM Sodium Citrate, pH 3.5 / Acetonitrile (25/75)
Sample:	2 µg/ml Neuromedin in Buffer
Injection:	500 seconds at 8 KV
Elution:	20 seconds at 7KV
Run:	30 minutes at 8 KV

Migration Time	Area
26.13	90392
25.92	89711
25.60	89531
25.41	92269
25.20	91398
mean = 25.65	90,660
S.D. = 0.336	1037
%R.S.D = 1.14	1.30

EFFECT OF CAPILLARY INSIDE DIAMETER ON PLATE COUNT





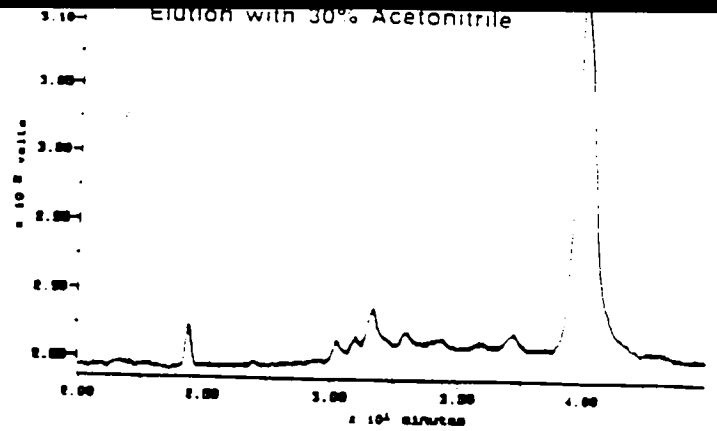
In order to examine possible sample carry-over, a second elution with organic solvent was performed. No additional peaks were observed.

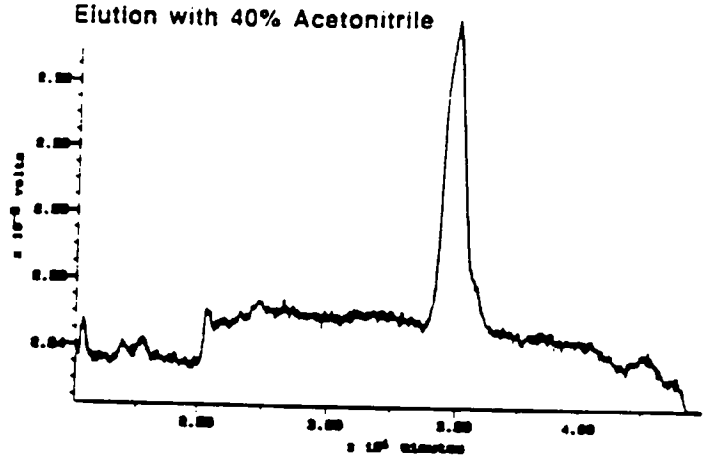
Conditions:

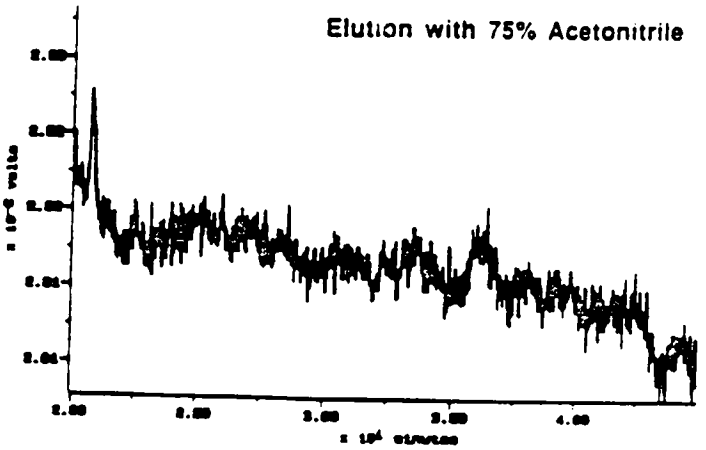
Capillary:	75 µm x 60 cm, 0.6 mm Delta Pak™
Buffer:	25 mM Na Citrate, pH 3.5
Eluent:	25 mM Na Citrate, pH 3.5/ Acetonitrile 25/75
Sample:	2 µg/ml Neuromedin in Buffer
Injection:	100 seconds at 12 kV
Elution:	10 seconds at 7 kV
Run:	12 kV

Extending the pH Range for Pre-Concentration

Our first experiments employed a silica based packing material for pre-concentration. This limits the pH range to 3-7. In order to increase the utility of this technology, we next used a polymeric hydrophobic packing material. This extends the pH range to 3-10. The properties of this capillary are otherwise very similar to the silica based capillary.



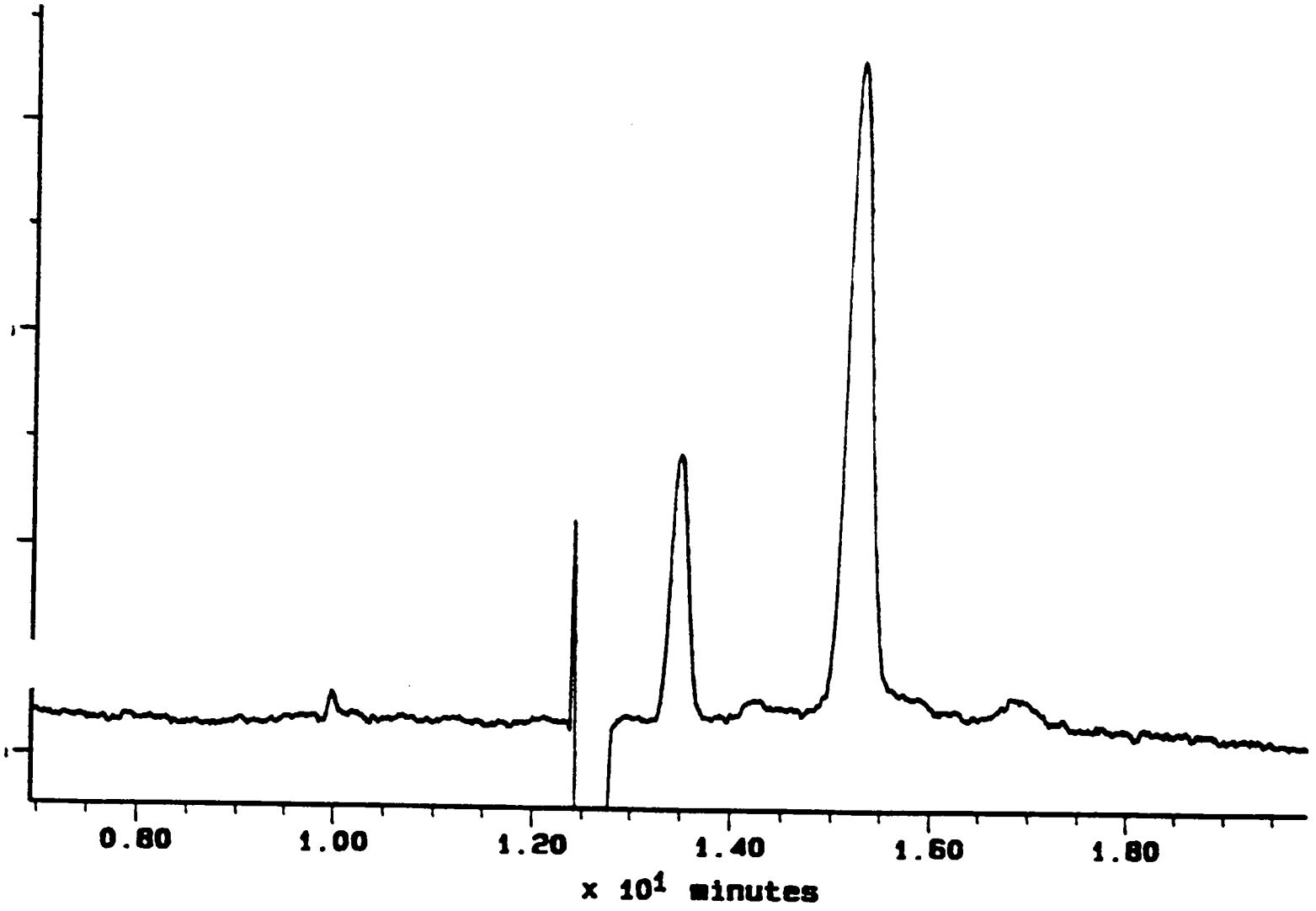




Conditions:

Capillary:	100 µm x 60 cm, 1.5 mm Delta Pak™
Buffer:	25 mM Na Citrate, pH 4.0
Eluent:	25 mM Na Citrate/Acetonitrile Ratio As Indicated
Sample:	10 µg/ml Tryptic Digest Cytochrome c in Buffer
Injection:	500 seconds, 8 kV
Elution:	20 seconds, 7 kV
Run:	8 kV

ANALYSIS OF ANGIOTENSIN I AT pH 10.5

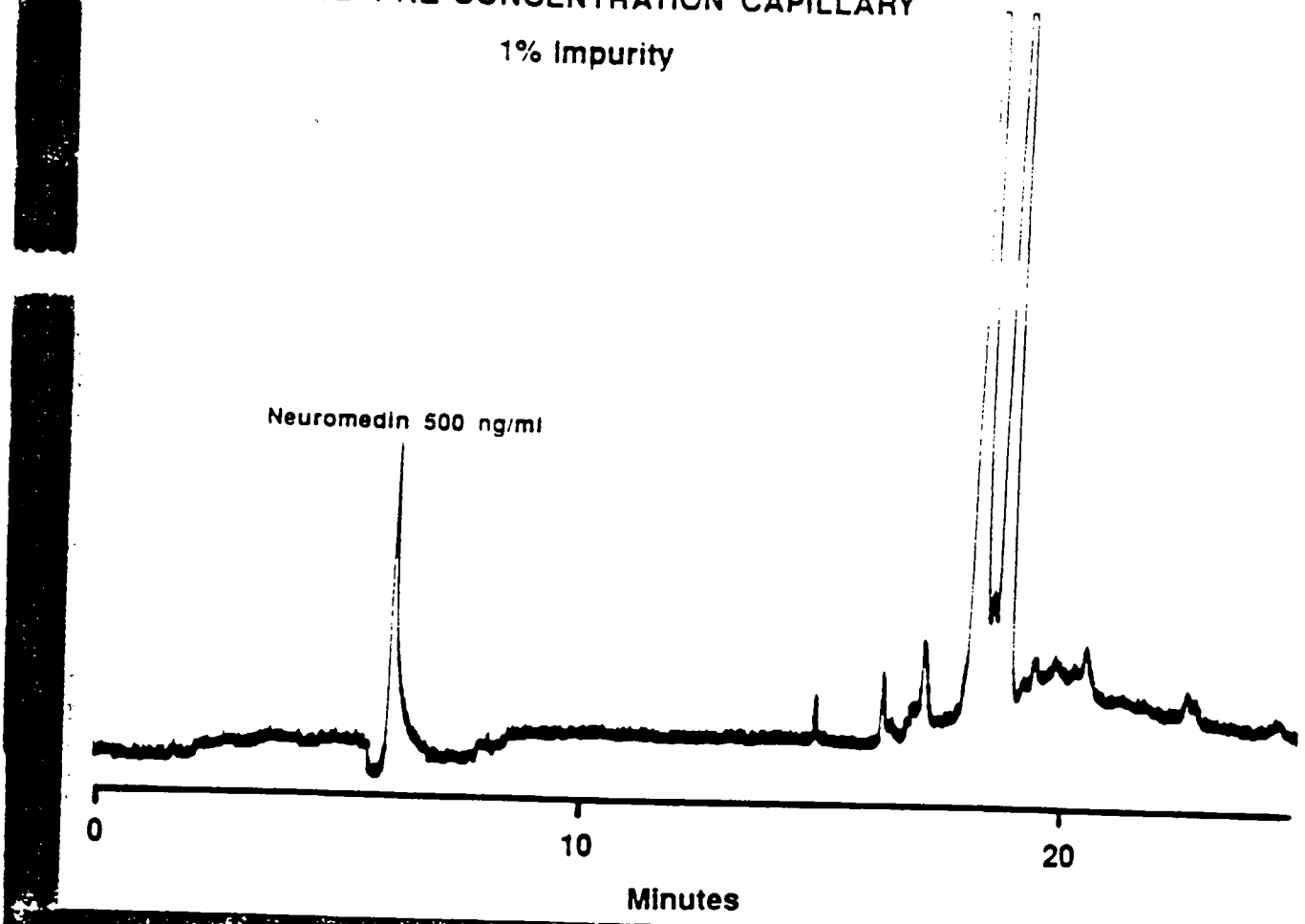


Conditions:

Capillary:	75 µm x 60 cm. 1 mm Polymeric Packing
Buffer:	50 mM Na Borate, pH 10.5
Eluent:	50 mM Na Borate, pH 10.5/Acetonitrile 25/75
Sample:	1 µg/ml Angiotensin I in Buffer + 1M AccuPure™ Z1 Methyl
Injection:	999 seconds at 15 kV
Elution:	10 seconds at 7.5 kV
Run:	15 kV

DETECTION OF TRACE IMPURITIES USING PRE-CONCENTRATION CAPILLARY

1% Impurity



Conditions:

Capillary:	75 µm x 60 cm, 0.6 mm Delta-Pak™
Buffer:	25 mM Na Citrate, pH 4.0
Eluent:	25 mM Na Citrate, pH 4.0/Acetonitrile 25/75
Sample:	500 ng/ml Neuromedin + 50 µg/ml Angiotensin II in Buffer
Injection:	500 seconds at 12 kV
Elution:	10 seconds at 7 kV
Run:	12 kV

CONCLUSIONS

* Adding On-Line Sample Concentration to Capillary Electrophoresis Can Greatly Increase Sensitivity With Respect To Sample Concentration. 250x Demonstrated In This Paper.

* The Pre-Concentration Capillary Behaves in a Linear Fashion with Respect to Sample Injection.

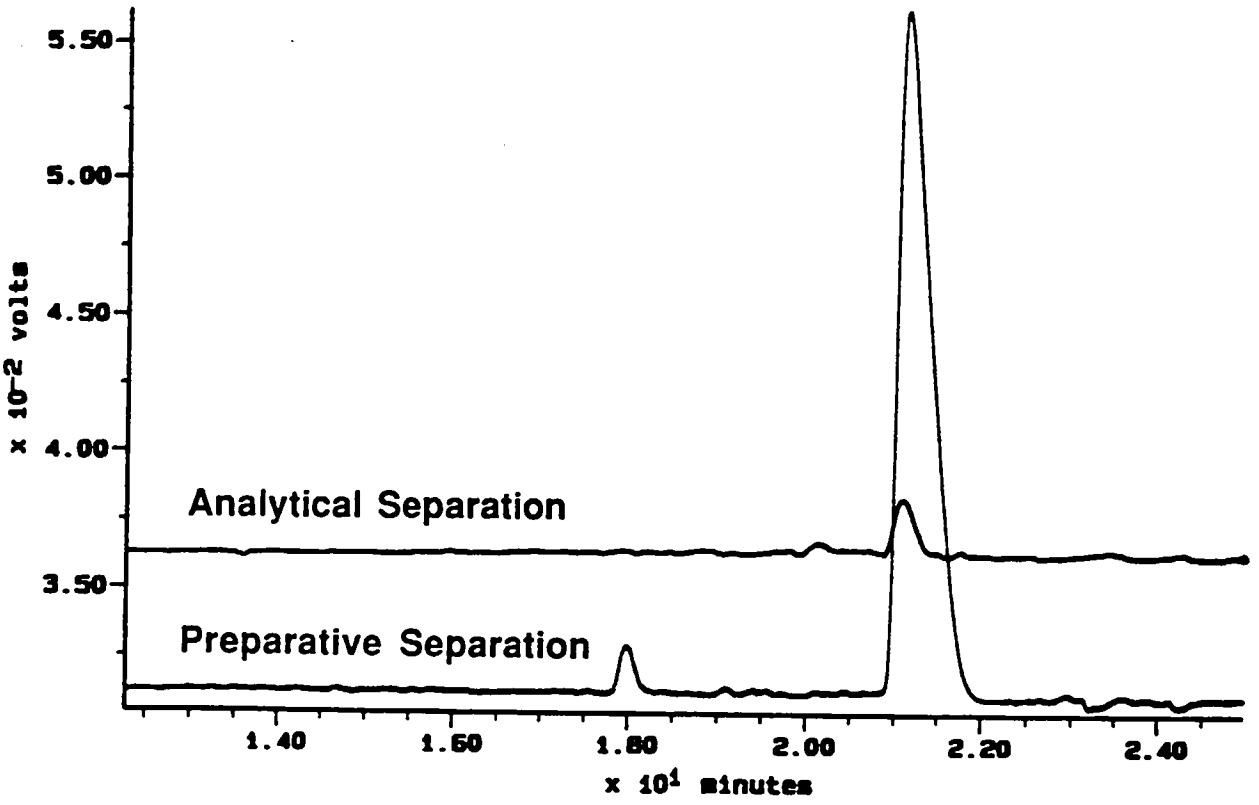
* Separations Using the Pre-Concentration Capillary are Reproducible with Respect to Migration Time and Peak Area.

* Separations Using the Pre-Concentration Capillary Result in High Resolution

* Using the Pre-Concentration Capillary, Contaminants May Be Detected Well Below the 0.02% Level.

* The Use of Step Gradient Elutions With the Pre-Concentration Capillary Can Result In 2 Dimensional Separations.

USE OF PRE-CONCENTRATION CAPILLARY FOR CE FRACTION ANALYSIS



Conditions: Preparative Separation

Capillary:	75 µm x 60 cm AccuSep™ Fused Silica Capillary
Buffer:	25 mM Na Citrate, pH 4.0
Sample:	200 µg/ml Angiotensin I in Buffer
Injection:	20 seconds, Hydrostatic
Run:	15 kV

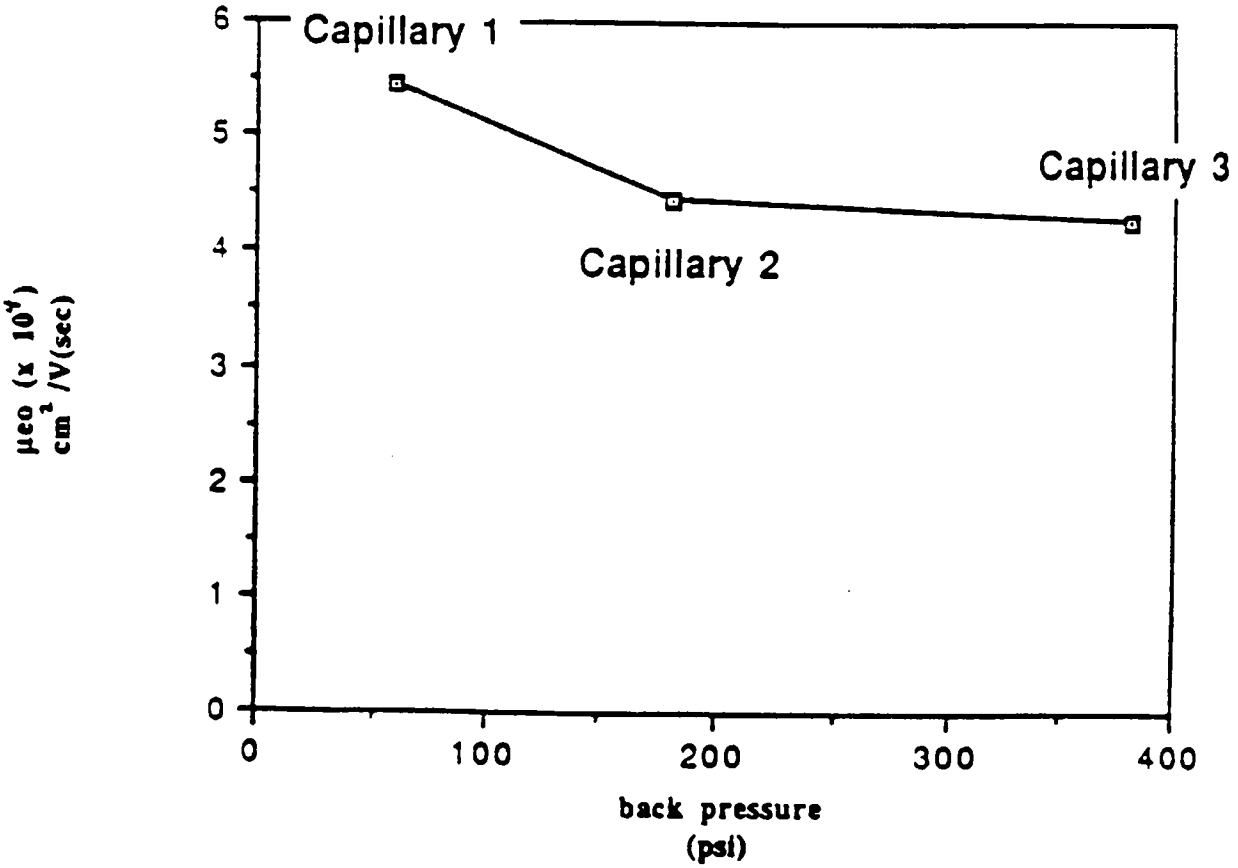
Conditions: Fraction Analysis

Capillary:	75 µm x 60 cm. 1 mm Polymeric Packing
Buffer:	25 mM Na Citrate, pH 4.0
Eluent:	25 mM Na Citrate, pH 4.0/Acetoni 25/75
Sample:	Collected Fraction + 1M AccuPur Z1 Methyl
Injection:	999 seconds at 15 kV
Elution:	10 seconds at 7.5 kV
Run:	15 kV

USE OF THE PRE-CONCENTRATION CAPILLARY FOR ANALYSIS OF CE FRACTIONS

It is possible to use Capillary Electrophoresis as a preparative technique. However, because small sample masses (1 pg- 1 ng) are collected in several μls of buffer, the concentration of the collected fraction is generally too low to be further analyzed using CE. The use of the pre-concentration capillary enables the user to analyze CE fractions directly.

INFLUENCE OF BACK PRESSURE ON ELECTROOSMOTIC FLOW



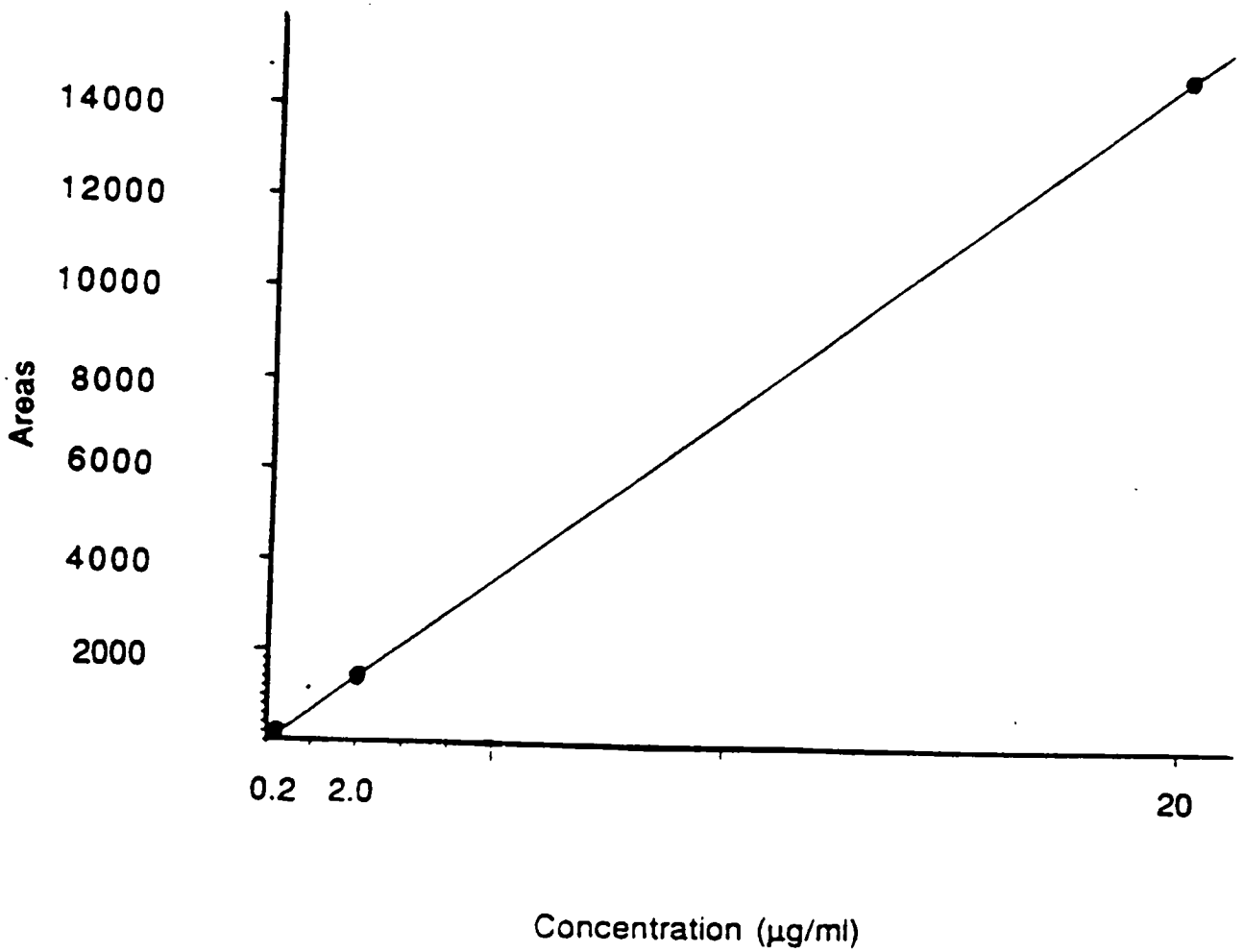
In order to determine the impact of the packing material on the rate of osmotic flow, 3 capillaries with dimensions of 100 μm x 60 cm were tested. The pack pressure was determined using an HPLC pump with a flow rate of 100 μl/minute. The rate of osmotic flow was determined using a neutral marker.

Capillary 1: No packing material
Capillary 2: 1 mm packed bed of Delta-Pak™
Capillary 3: 4 mm packed bed of Delta-Pak™

Linearity Using A Preconcentration Capillary

Experimental:

Capillary:	100 µm x 50 cm , 0.66 mm Delta-Pak
Buffer:	25 mM Sodium Citrate, pH 3.5
Eluent:	12.5 mM Sodium Citrate, pH 3.5 / Methanol (50/50)
Sample:	2 µg/ml Neuromedin in Buffer
Injection:	500 seconds at 5 KV
Elution:	20 seconds at 7KV
Run:	10 KV



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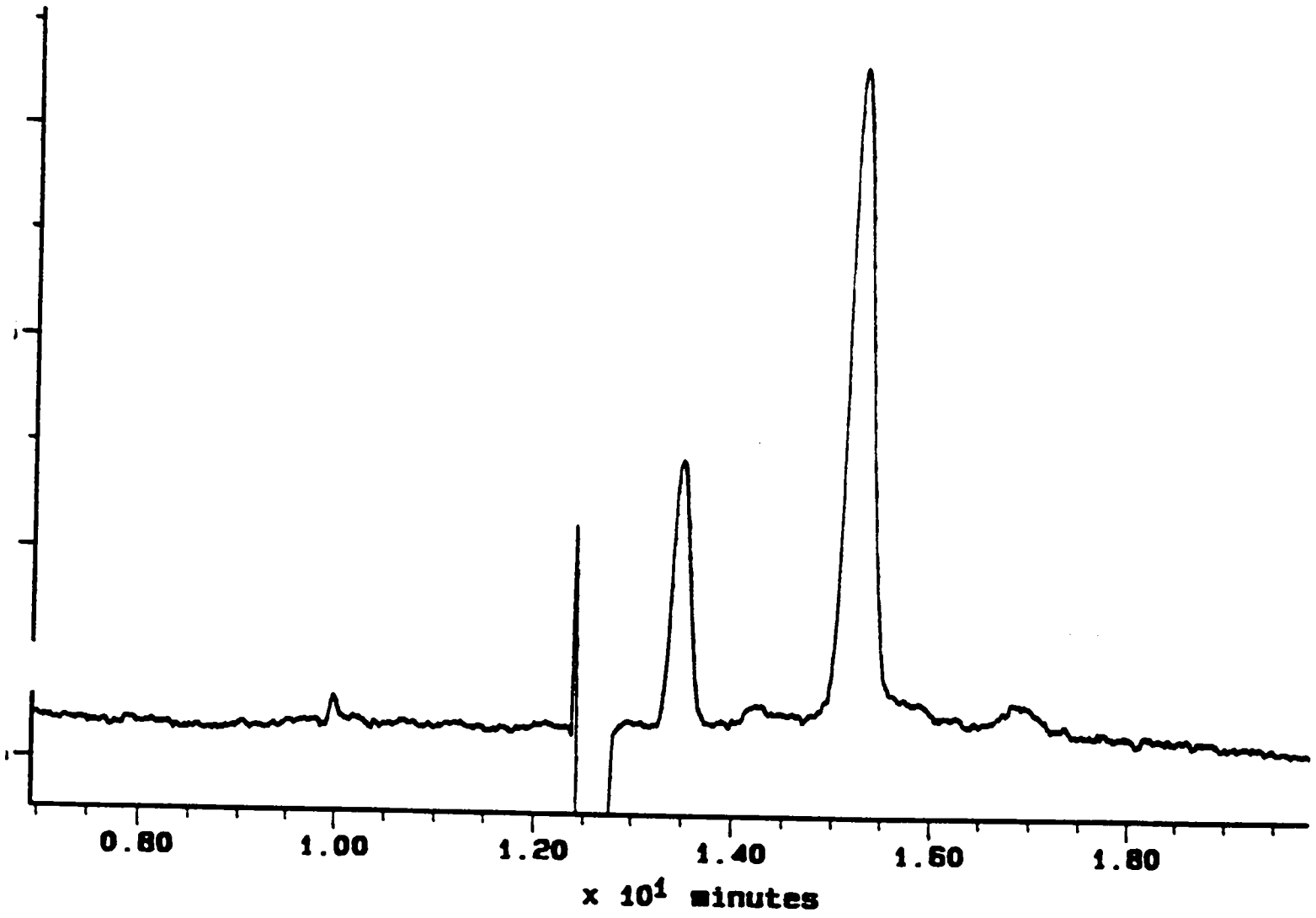

Conditions:

Capillary:	75 µm x 60 cm. 1 mm Polymeric Packing
Buffer:	50 mM Na Borate, pH 10.5
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Sample:	1 µg/ml Angiotensin I in Buffer + 1M AccuPure™ Z1 Methyl
Injection:	999 seconds at 15 kV
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