Feasibility of Whole-Fiber Cotton Columns for Study of Penetration of Solutes into Cotton Cellulose

SEA-USDA Cooperative Agreement
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Final Report

by

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and

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Introduction

Briefly, this project involved an examination of the feasibility of high-performance liquid chromatography (HPLC) to study the penetration of solutes into whole cotton fiber. Rowland and associates have previously used open-bed chromatography with columns packed with cotton fiber. It was speculated that improved chromatographic performance would be achieved using HPLC type columns in view of their improved resolution in ordinary chromatographic separations.

Radial-pak cartridges were to be packed by Waters Associates with

1. Caustic-scoured with cotton fibers
2. Ammonia mercerized whole cotton fibers, using fiber that had been previously caustic scoured
3. NaOH mercerized whole cotton fibers, using fiber that had been previously caustic scoured.

Each column was to be characterized using a variety of solutes including (a) oligomeric sugars, (b) members of the ethylene glycol-polyethylene glycol series, (c) glymes, (d) selected N-methylol reagents employed in the durable-press finishing of cotton-containing fabrics.

Experimental

A Waters Associates ALC 204 Liquid Chromatograph equipped with a Model 6000 A Solvent Delivery System, a U6K Universal Injector, a Model R-401 Differential Refractometer, a Model RCM-100 Radial Compression Module, and a Fisher Recordall Series 500 Recorder was employed Radial-Pak Compression Cartridges were packed with various samples of whole cotton fiber by Hubert Quinn of Waters Associates.

Solution of the following compounds and concentrations were prepared using HPLC purified water:

	Compound	Concentration
a)	Oligomeric Sugars:	
	α-D(+) Glucose	0.025 g/ml
	Maltose	0.020 or 0.025 g/ml
	Maltotriose	0.020 or 0.025 g/ml
	D(+) Ralfinose	0.025 g/ml
	Stachyose	0.015 g/ml
b)	Glymes:	
	Diglyme	0.025 ml/ml
	Tetraglyme	0.025 ml/ml
c)	Polyethylene Glycols:	
	PEG-200	0.025 ml/ml
	PEG-550	0.025 g/ml
	PEG-3350	0.025 g/ml
	PEG-4000	0.025 g/ml
	PEG-6000	0.025 g/ml

The HPLC operating conditions were as follows:

solvent: HPLC purified water

solvent flow rate: 1.0 ml/min

chart speed: 1.0 cm/min

R-401 attenuator: 4x

These conditions were used throughout this study unless otherwise stated. Five µl samples of each of the abovementioned solutions were injected onto the column and the retention times recorded.

Results and Discussion

The first trials used a Radial-Pak cartridge packed with caustic scoured cotton fiber. (The raw fiber was supplied by Dr. S. P. Rowland.)

This cartridge collapsed almost immediately as too high a pressure was applied with the RCM-100 module. Another sample of more mature cotton fiber was obtained from Dr. Rowland, caustic scoured, and sent to Waters Associates for preparation of a second cartridge. Dr. Quinn recommended that only a limited pressure be applied.

This column showed very poor discrimination as shown by the retention times in Table I for the various polyhydroxyl compounds of different molecular weight. Changing the solvent flow rate to 1.5 ml/min improved the peak resolution but there was no real improvement in discrimination (see Table I).

Undoubtedly, during these runs there was compaction of the cotton in the column as indicated by a continuous increase in pump back pressure from ≃ 50 psig up to ≃ 4500 psig during the runs. Increasing the pressure on the REM-100 module might prevent compaction but it would cause collapse of the cartridge.

The above column was packed with cotton fibers with the fibers in more-or-less parallel array and parallel with the long dimension of the cartridge. Dr. Rowland had greater success with open bed columns prepared by punching out discs, of appropriate site, from cotton laps. After consultation with him it was decided to pack a Radial-Pak cartridge in a similar fashion. A sample of Rexall Drug Company sterilized cotton "batting" was obtained and approximately 0.5 cm diameter discs were punched out using a hole punch. The discs were then packed at Waters Associates into a Radial-Pak Cartridge by placing the dry discs one on top of the other in the column.

As the discs were dry it was necessary to pump water thru the column at a constant rate to completely wet-out the fibers. It required

nearly seventy hours until a stable baseline on the refractometer was obtained.

This column did not exhibit any pressure build-up during continuous use as encountered with the previous column.

Retention times for the various polyhydroxyl compounds are summarized in Table II; typical chromatograms are shown in Figures 1-12. It will be noted that this column also demonstrated very poor discrimination. Three of the compounds were examined using a reduced flow rate (Table III) but no improvement in discrimination was achieved. The chromatograms are in Figures 13-15.

It is apparent from the chromatograms shown in Figures 1-15 that a strong "tailing" occurs, almost to the point of the appearance of a second peak. There are two possible explanations for this. One explanation is that there are areas of different packing density along the length of the column. If the cotton discs were inserted into the column so that they were not arranged in parallel layers (for example, a disc was partially displaced along the wall and therefore did not completely cover the column cross-section) there would be areas, along the walls of the cartridge, which are more densely packed than the interior of the column. Channelling could occur and tailing would result. A second reason is that channelling along the sides of the column may be occurring. If the cotton discs were very uniformly packed, but they were not the exact size of the inner diameter of the column, it is possible that extreme channelling could take place. The main peak of the chromatogram would then be the portion of the sample that has travelled along the sides of the column with the tail being that portion which actually travel through the cotton fiber discs. The

virtually identical retention times of the samples along with the fact that only a very low level of pressure on the column in the RCM-100 module can be used unfortunately lend some plausability to this explanation.

Figures 16 & 17 are chromatograms obtained using the first cotton fiber column. There is some tailing but not to the extent of that obtained with the disc packed column.

The most distressing aspect of this study is, however, the fact that the column shows no discrimination (Table II). Lowering the solvent flow rate did not improve the discrimination (Table III, Figures 13-51).

If it is assumed that there is no extreme channelling, as described above, and that we are actually dealing with size exclusion chromatography, it can be speculated that the pores in the cotton fibers are too small to permit any of the compounds to enter. Although this would appear to be very unlikely in view of the molecular size of α-D-glucose, it was decided to test this hypothesis using methanol. The chromatogram for methanol is shown in Figure 18; the retention time was the lowest of all the compounds tested in this study.

The other extreme is that the pores in the fibers are so large that none of the compounds are retained. This scarcely seems plausible in view of the poly(ethylene glycol) of molecular weight 6000 having a retention time identical to the one with a molecular weight of 200. This is also confirmed by Figure 19 which is the chromatogram of a mixture of the 6000 and 200 molecular weight poly(ethylene glycol), there is a single peak. In addition, the retention time for the poly(ethylene glycol) 6000 is nearly identical to that of the α-D-glucose.

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Increasing the radial pressure on the column by means of the RCM-100 module was also investigated. The chromatograms for glucose, raffinose and poly(ethylene glycol) 6000 with increased radial pressure are shown in Figures 20-23. The shape of the peaks was altered and the retention times lowered. There is rather definite evidence of a second peak, indicating the column is non-uniform. It is possible that under the increased radial pressure the column has begun to collapse and has assumed an ellipsoid shape with channels at the outer edges of the ellipsoid.

Figures 24-27 are chromatograms under reduced radial pressure. The retention times are further lowered indicating increased channelling.

These results indicate that HPLC, at least using the type of columns employed here, is not a feasible method to study the penetration of solutes into cotton fibers.

Table I

Retention Time Values

Compound	Retention Time (min) Flow Rate: 1.0 ml/min	1.5 ml/min
Poly(ethylene glycol) (M.W. = 200)	4.50	3.45
Poly(ethylene glycol) (M.W. = 550)	4.40	-
Poly(ethylene glycol) (M.W. = 3350)	4.35	3.10
Diglyme	-	3.20
Tetraglyme	-	3.15
α-D(+)Glucose	4.35, 4.90	2.95
Maltose	5.30	-
Maltotriose	5.00	-
D(+)Raffinose	4.65	2.90
Stachyose	5.10	-

Table II

Retention Time Values

Solvent Flow Rate: 1.0 ml/min.

Compound	Retention Time (min.)
Poly(ethylene glycol) (M.W. = 200)	3.95
Poly(ethylene glycol) (M.W. = 550)	3.90
Poly(ethylene glycol) (M.W. = 3350)	3.95
Poly(ethylene glycol) (M.W. = 4000)	3.90
Poly(ethylene glycol) (M.W. = 6000)	3.95
Diglyme	4.05
Tetraglyme	4.00
α-D(+)Glucose	4.15
Maltose	4.05
Maltotriose	3.95
D(+)Raffinose	3.95
Stachyose	4.00

Table III

Retention Time Values

Solvent Flow Rate: 0.5 ml/min.

Compound	Retention Time (min.)
α-D(+)Glucose	7.90
Maltotriose	7.85
Stachyose	7.80

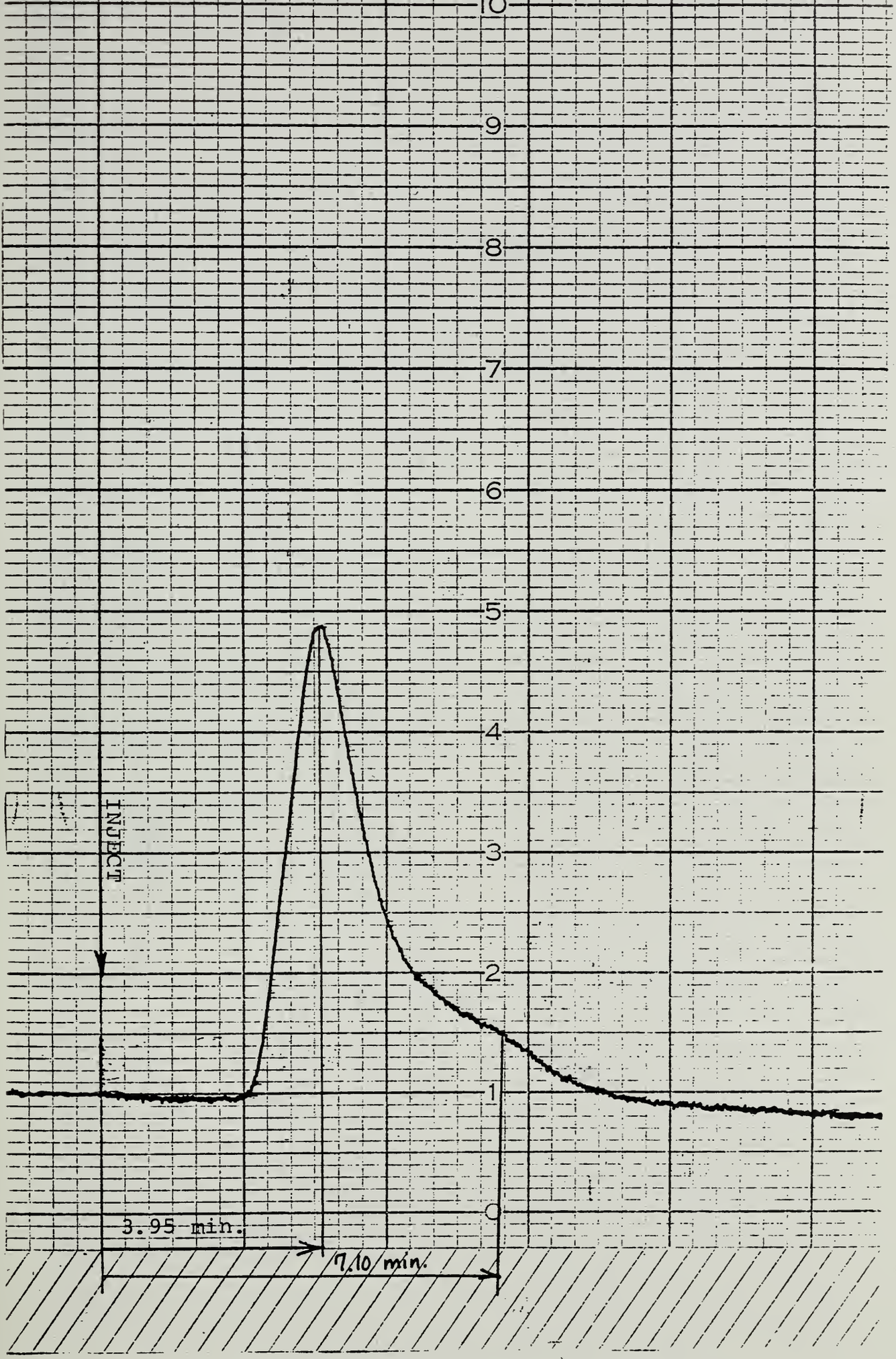

Figure 1. HPLC chromatogram of Polyethylene Glycol, molecular weight of 200.

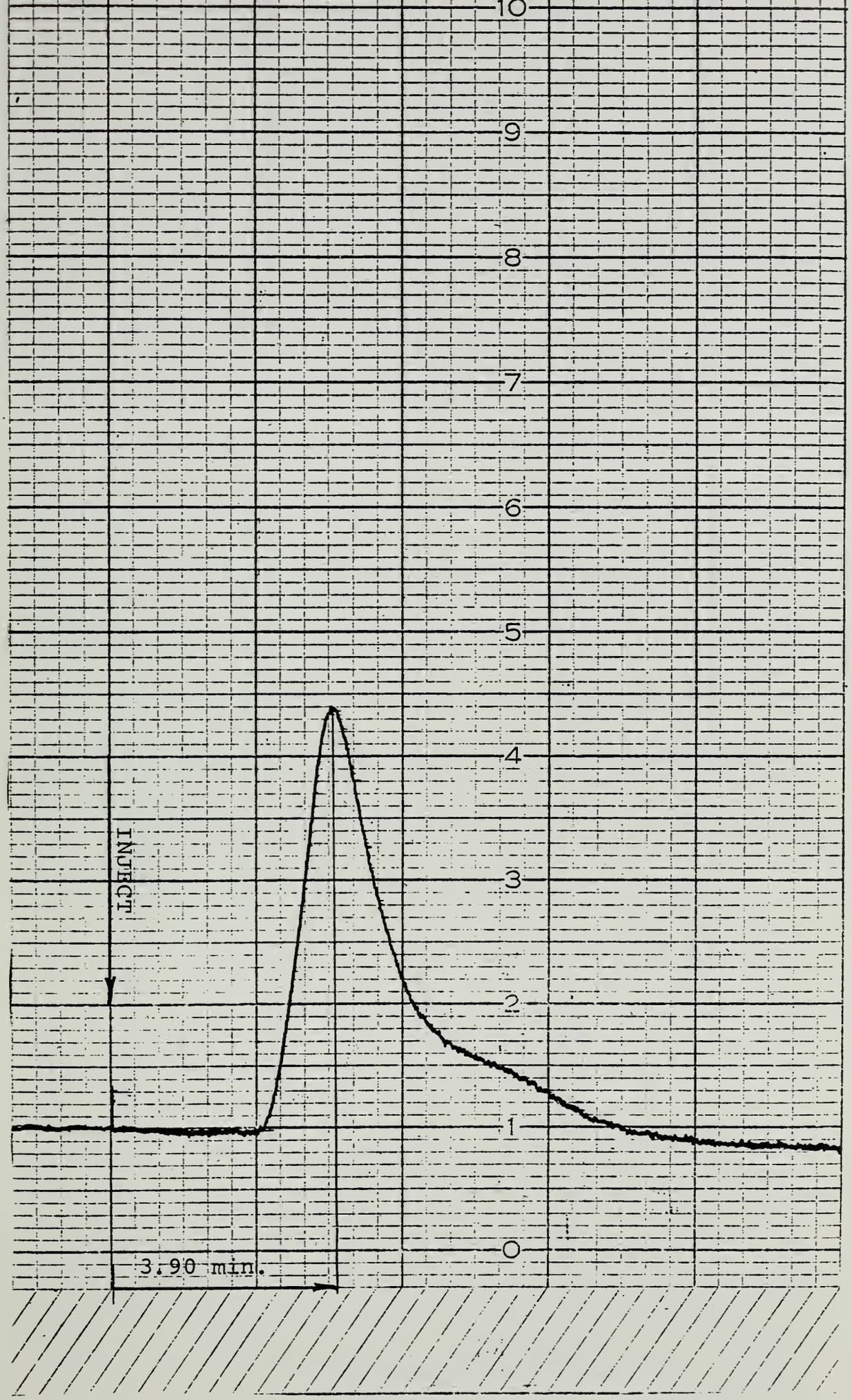

Figure 2. HPLC chromatogram of Polyethylene Glycol, molecular weight of 550.

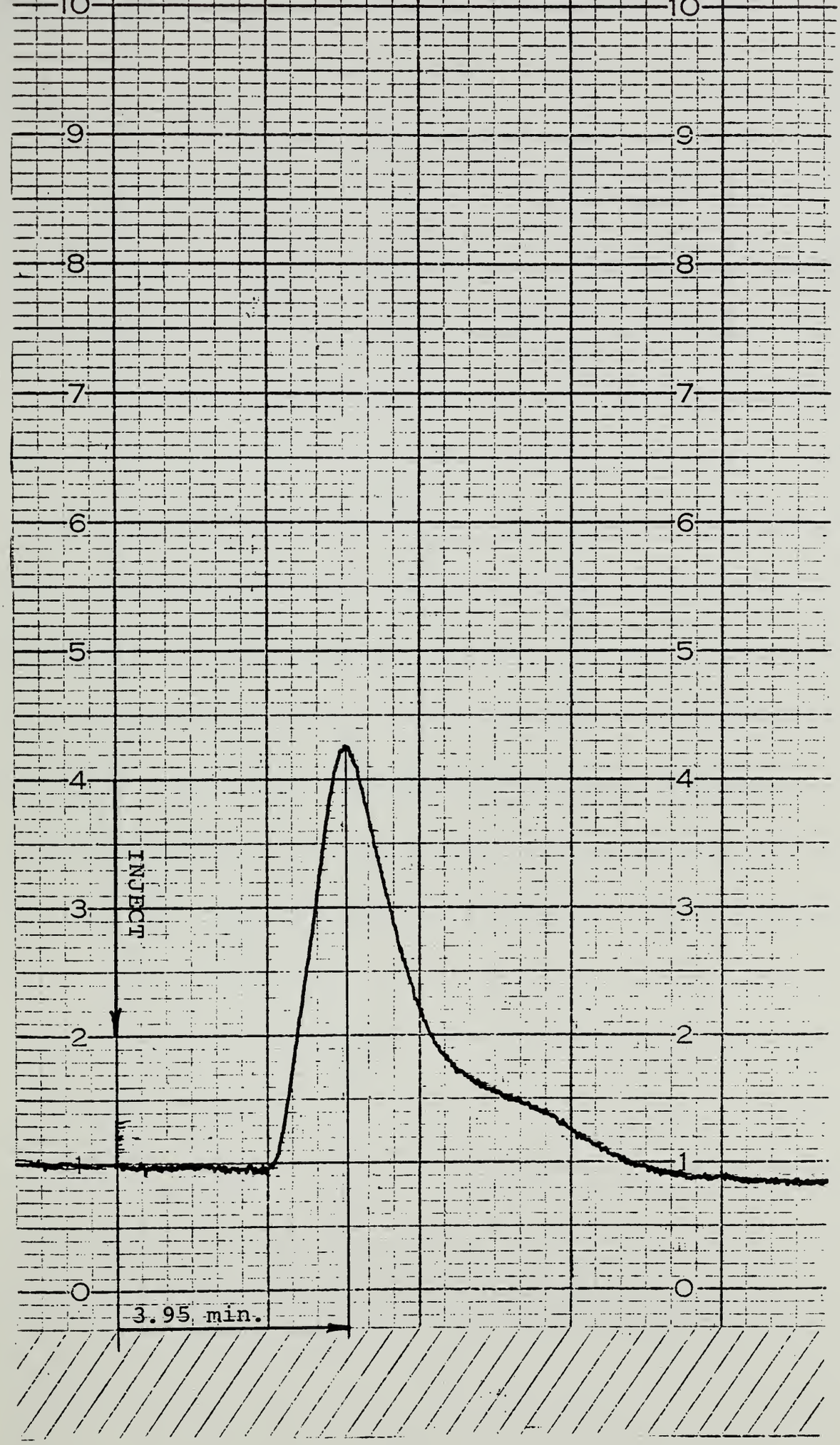

Figure 3. HPLC chromatogram of Polyethylene Glycol, molecular weight of 3350.

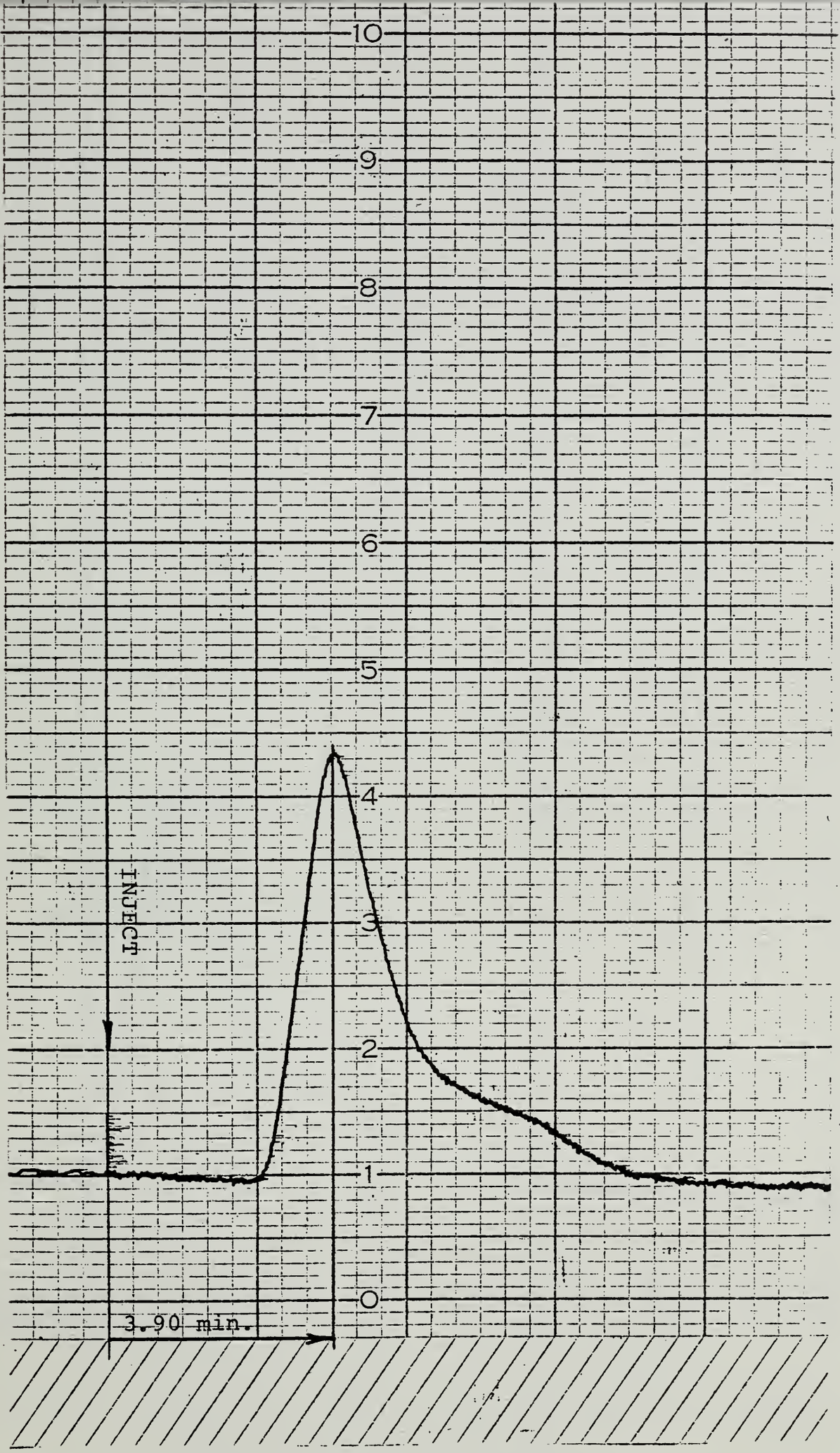

Figure 4. HPLC chromatogram of Polyethylene Glycol, molecular weight of 4000.

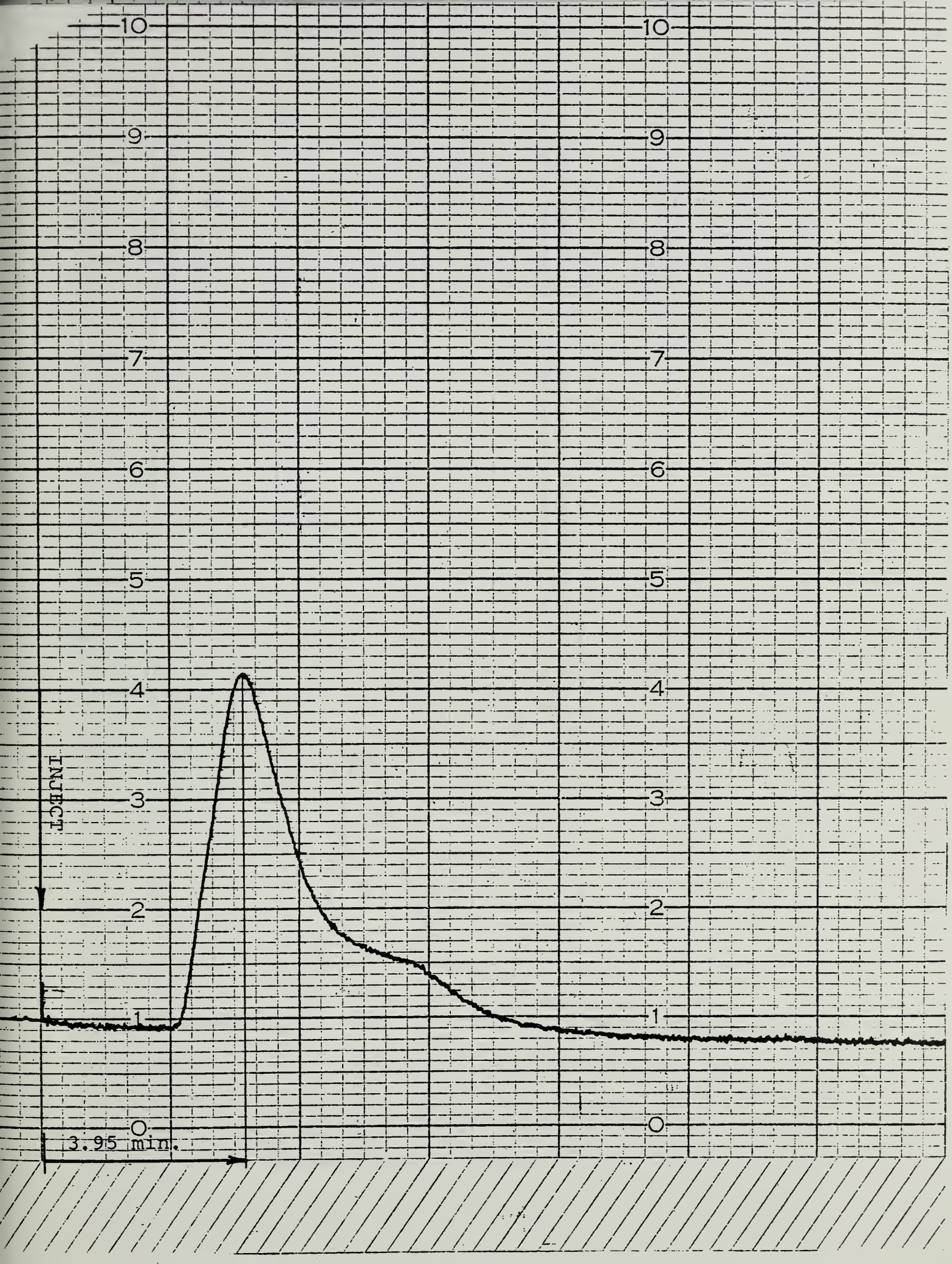

Figure 5. HPLC chromatogram of Polyethylene Glycol, molecular weight of 6000.

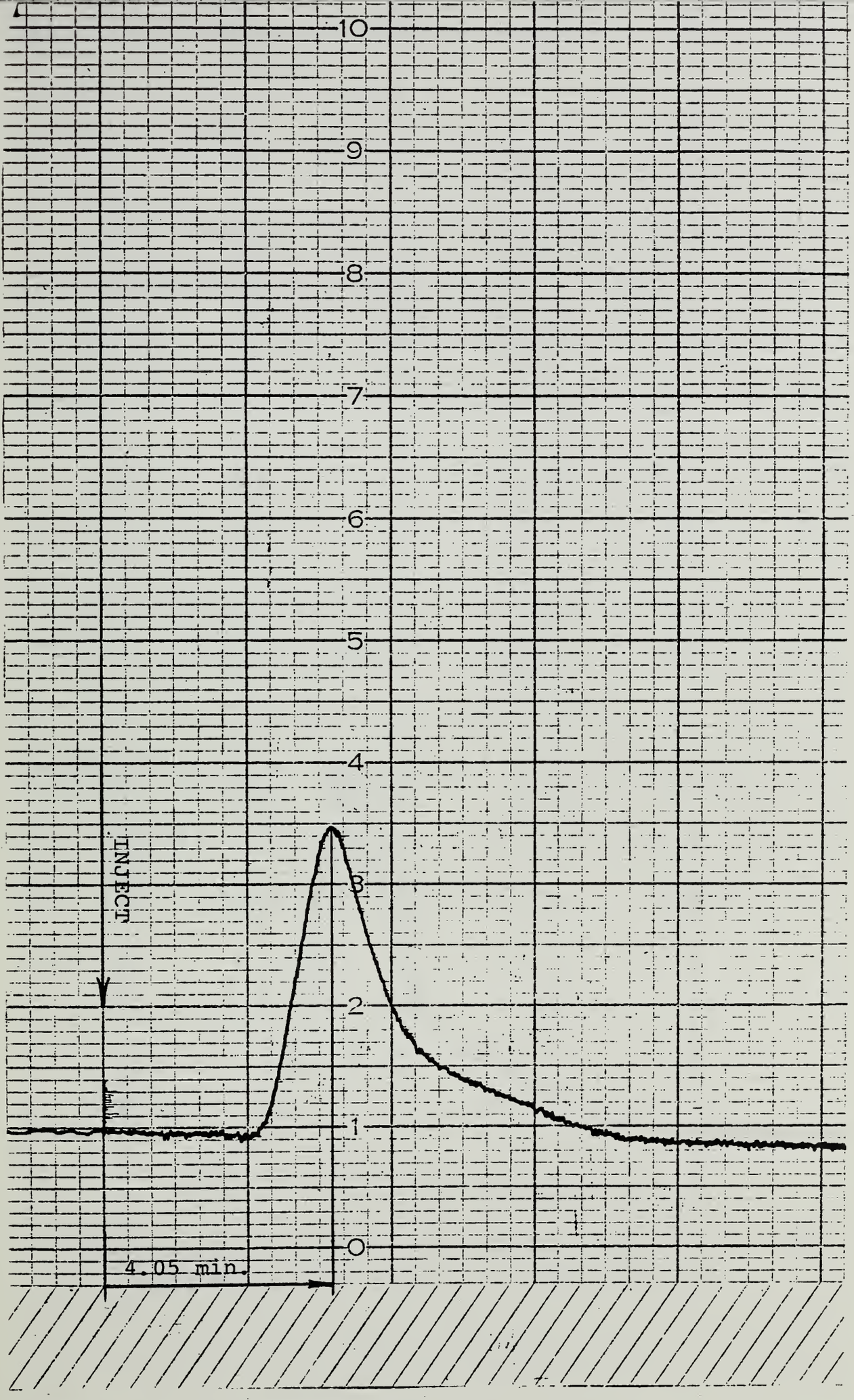

Figure 6. HPLC chromatogram of Diglyme.

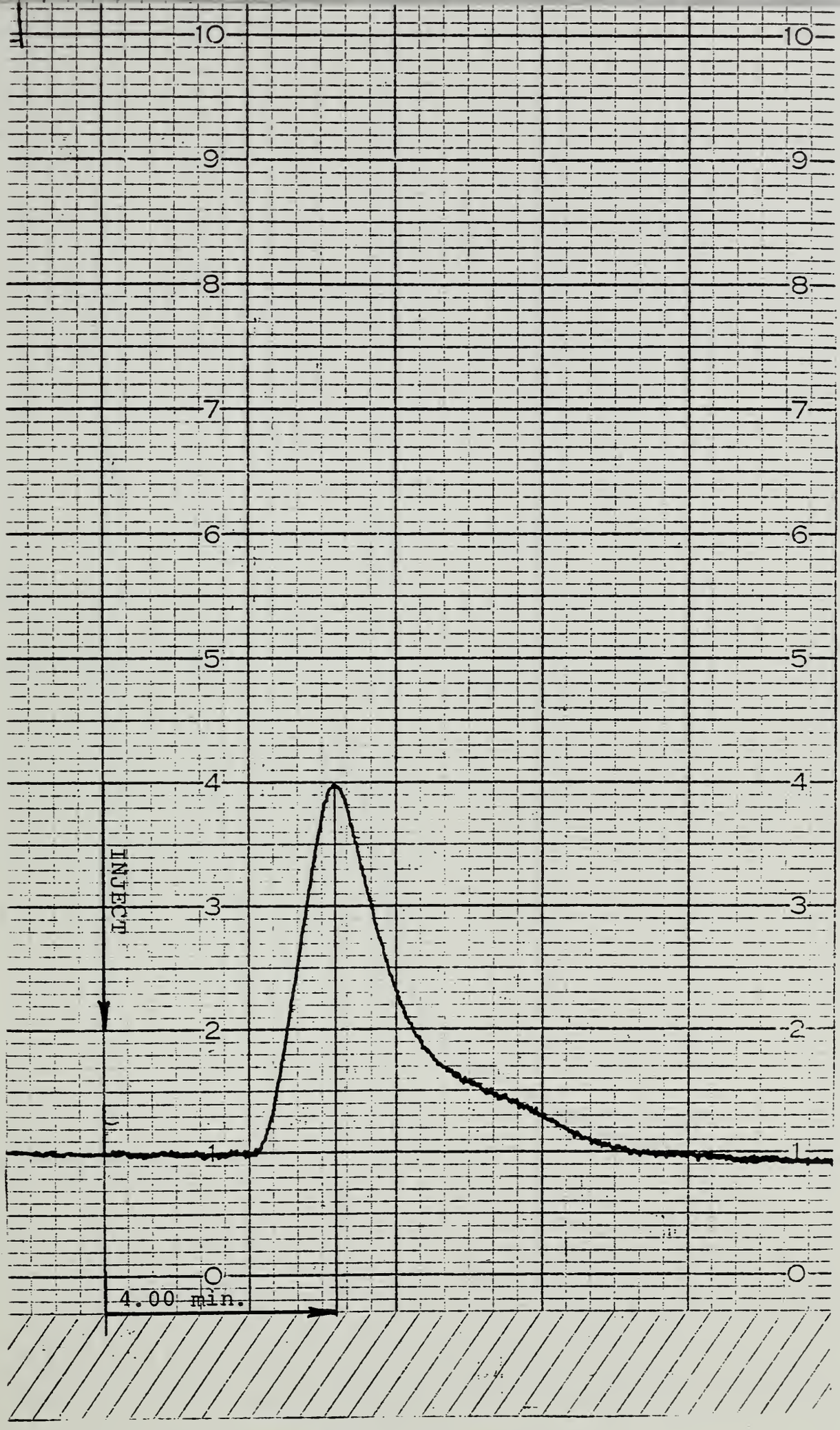

Figure 7. HPLC chromatogram of Tetraglyme.

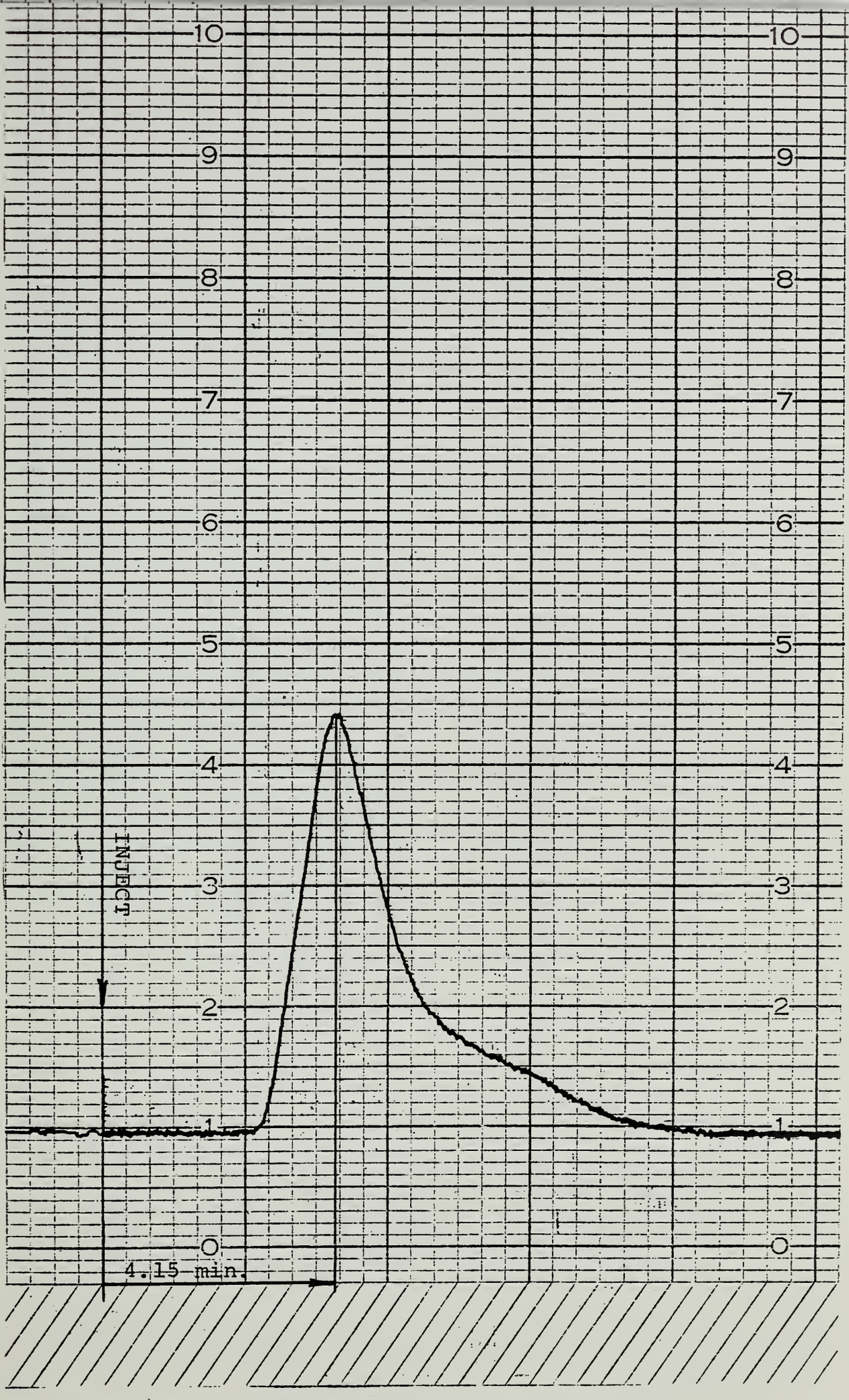

Figure 8. HPLC chromatogram of α-D(+)Glucose.

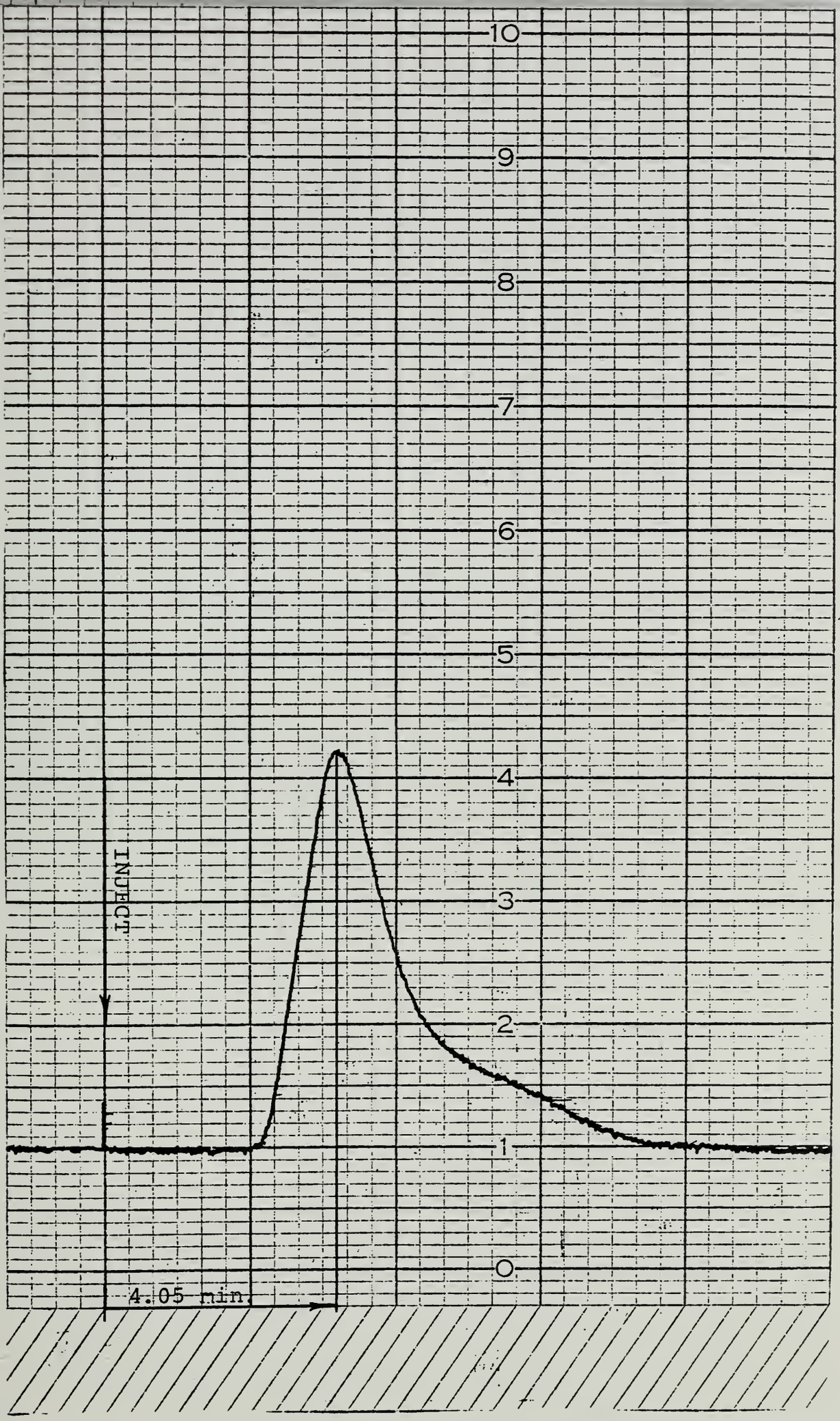

Figure 9. HPLC chromatogram of Maltose.

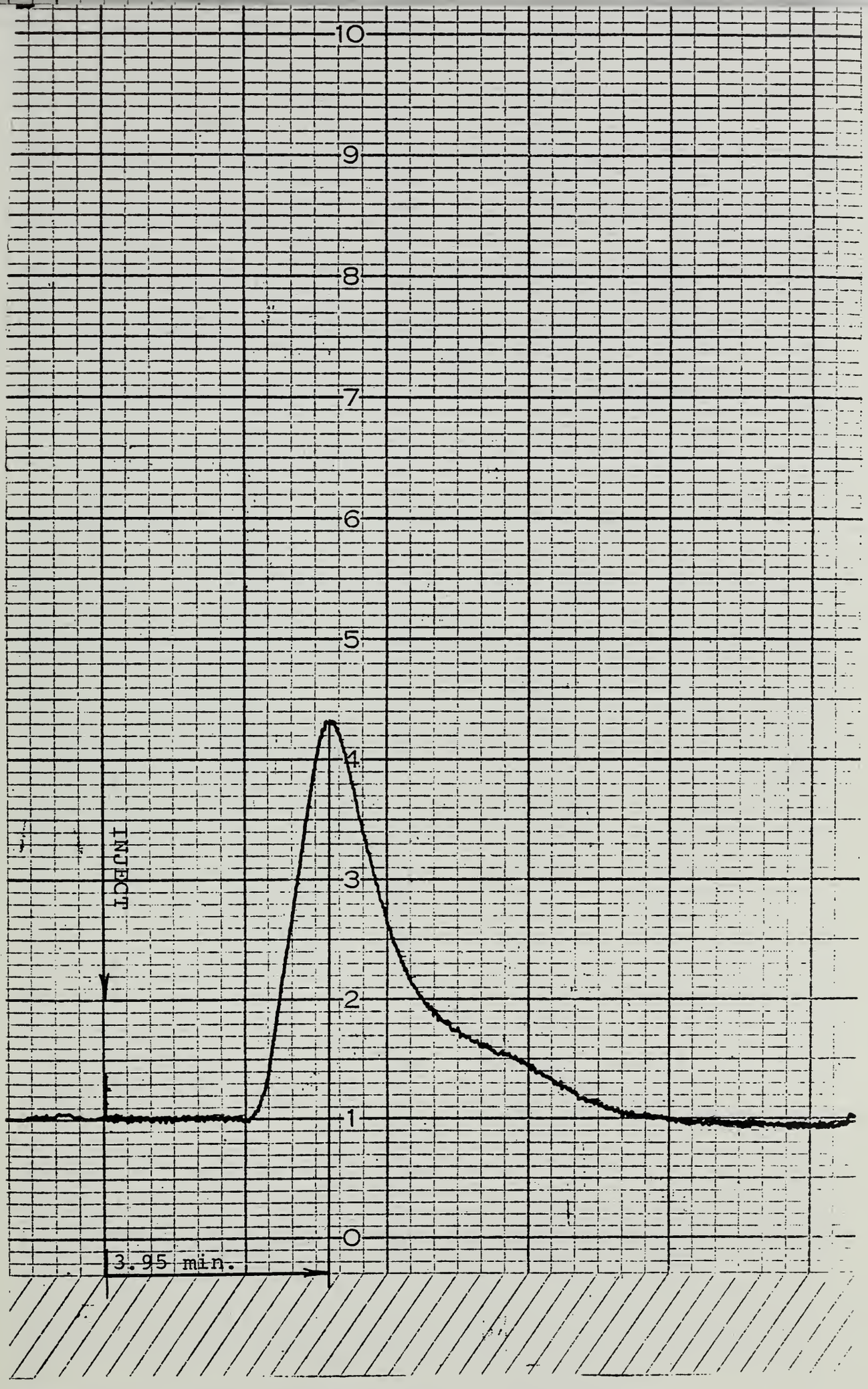

Figure 10. HPLC chromatogram of Maltotriose.

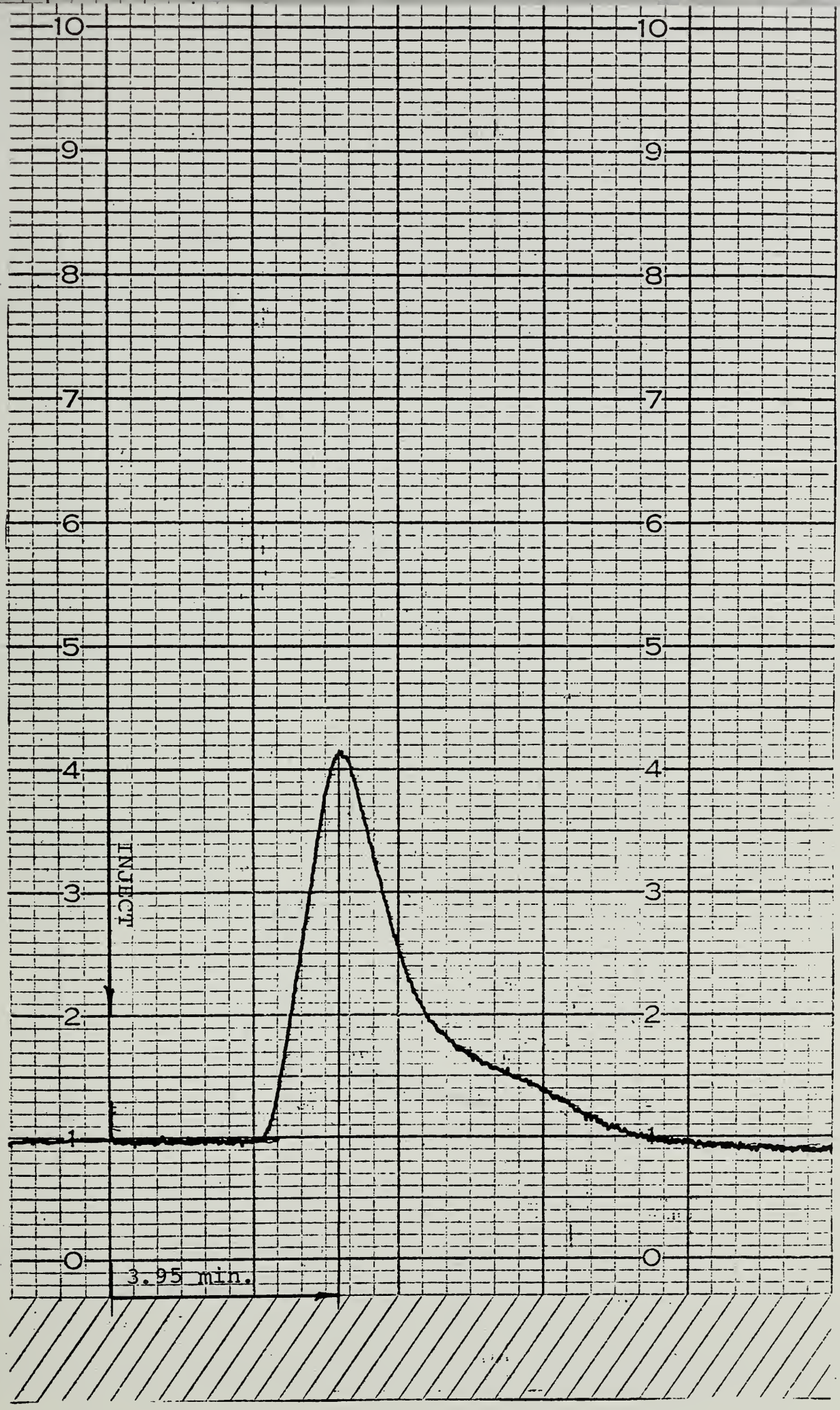

Figure 11. HPLC chromatogram of D(+)Raffinose.

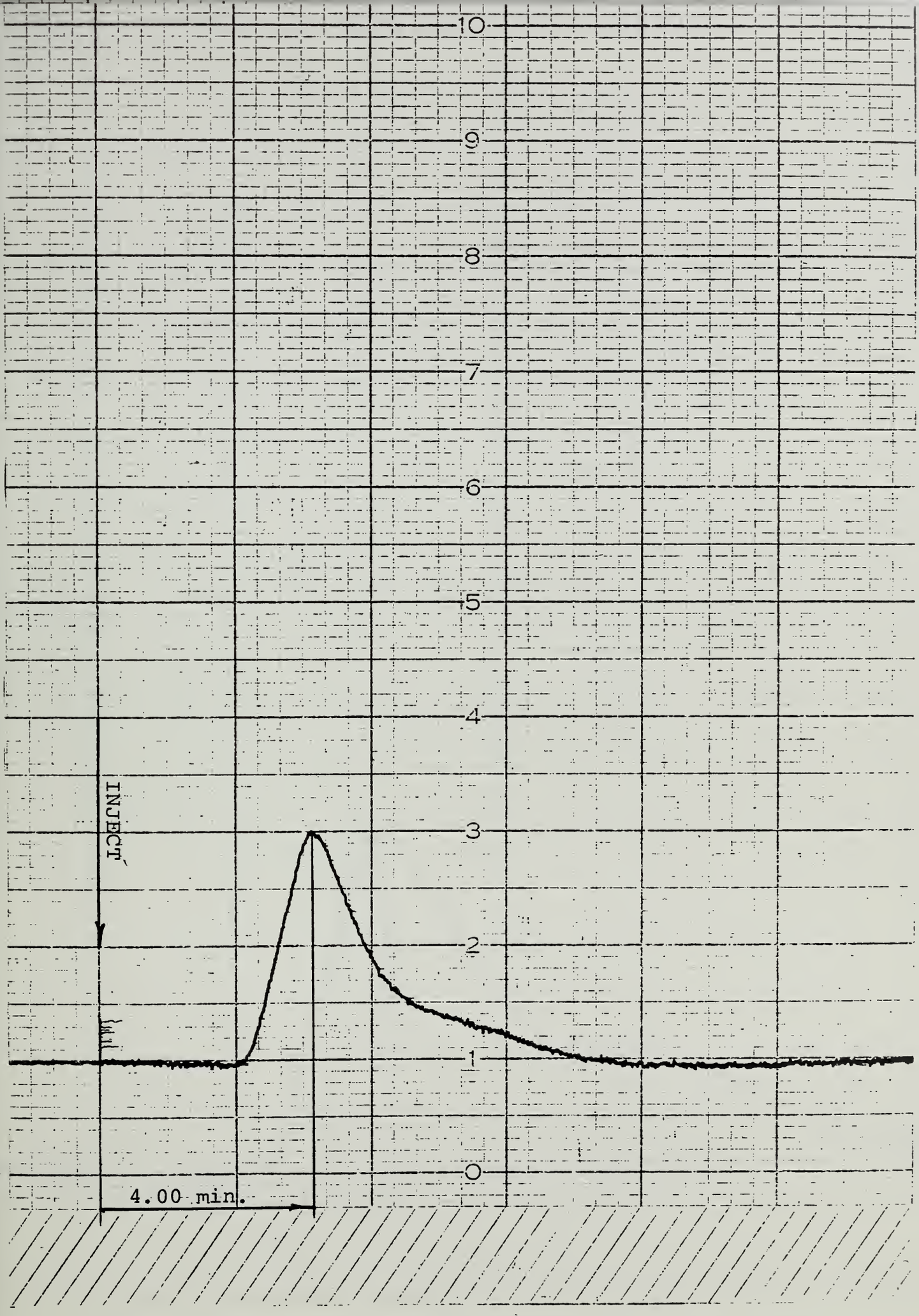

Figure 12. HPLC chromatogram of Stachyose.

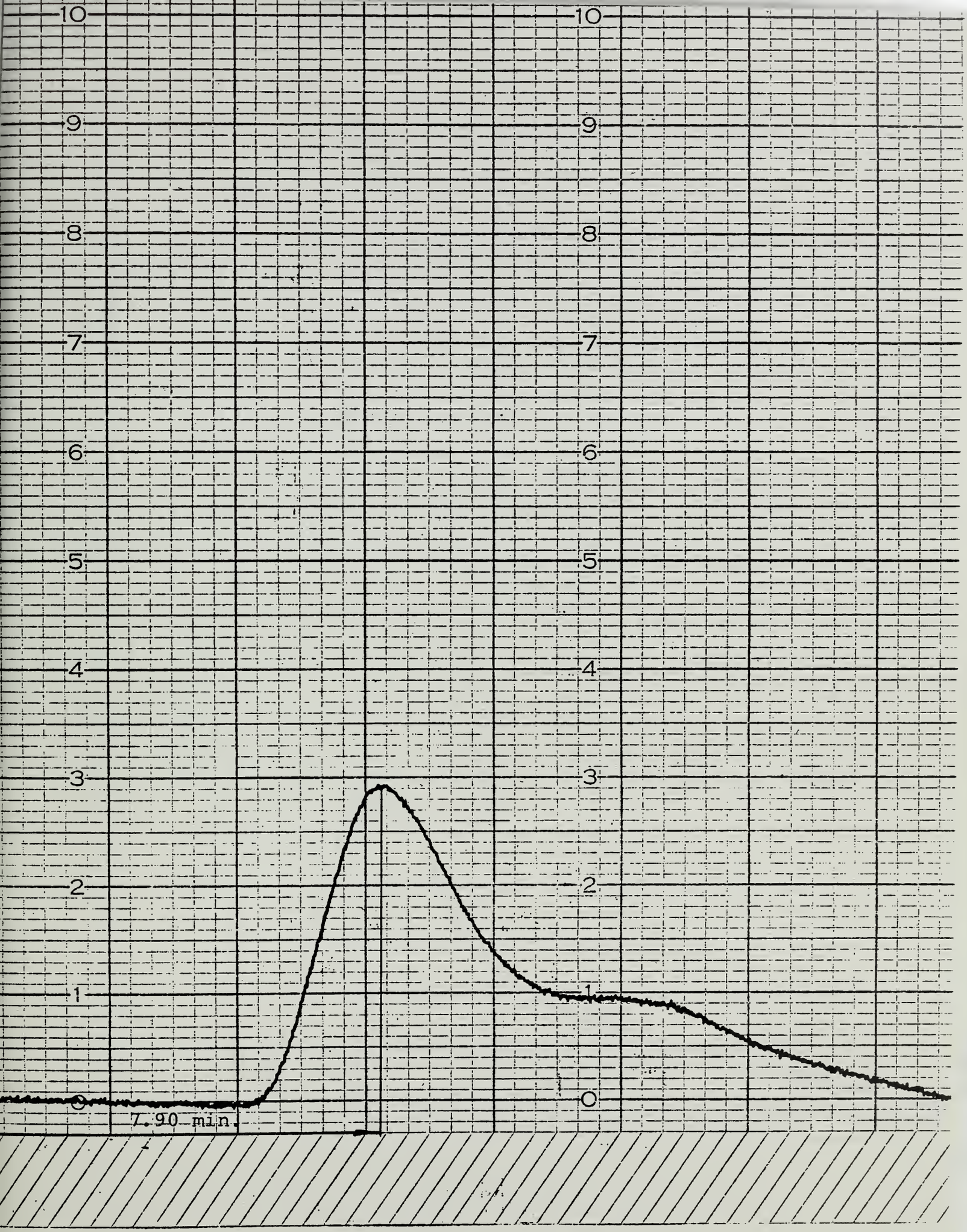

Figure 13. HPLC chromatogram of Glucose, at a flow rate of 0.5 ml/min.

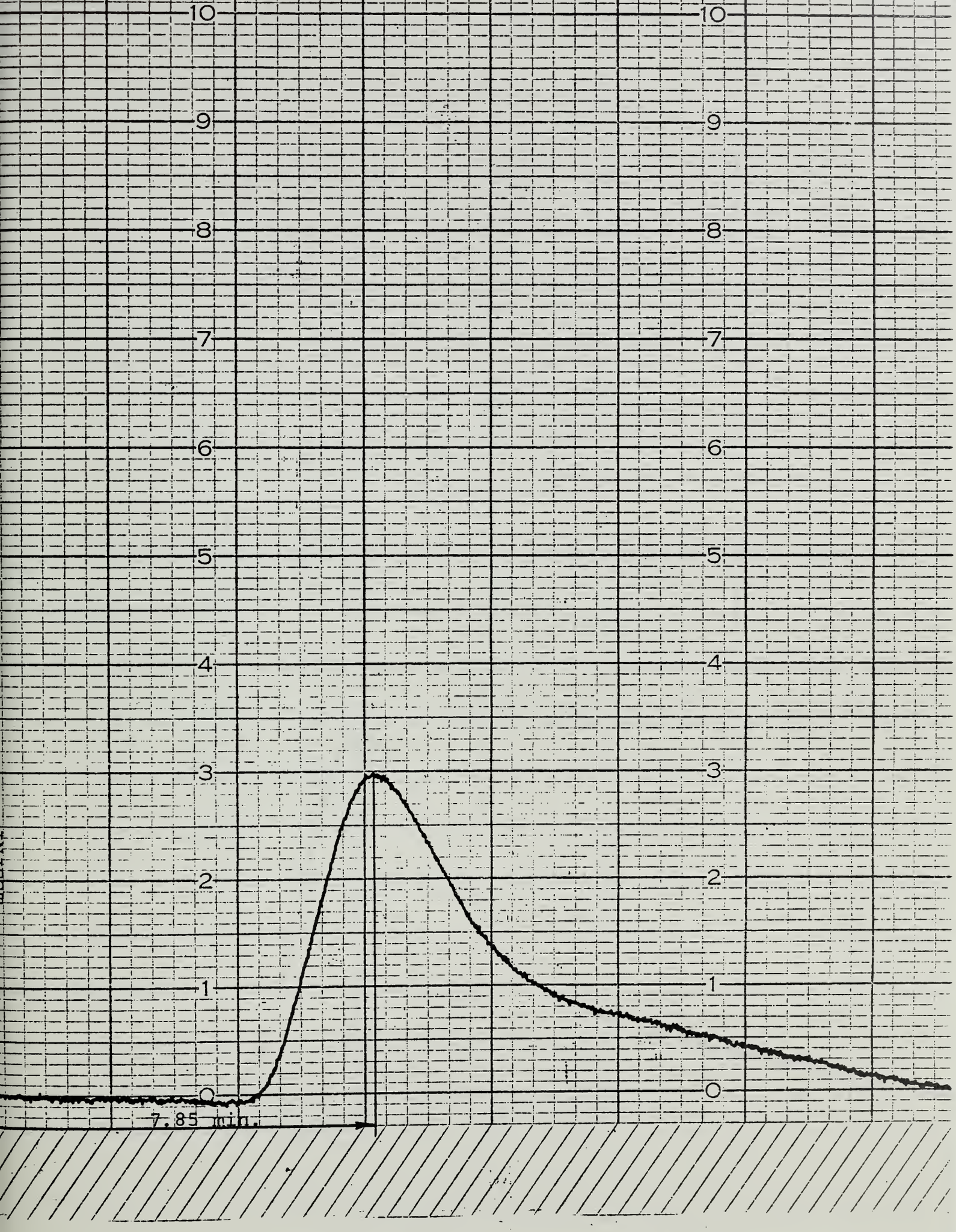

Figure 14. HPLC chromatogram of Maltotriose, at a flow rate of 0.5 ml/min.

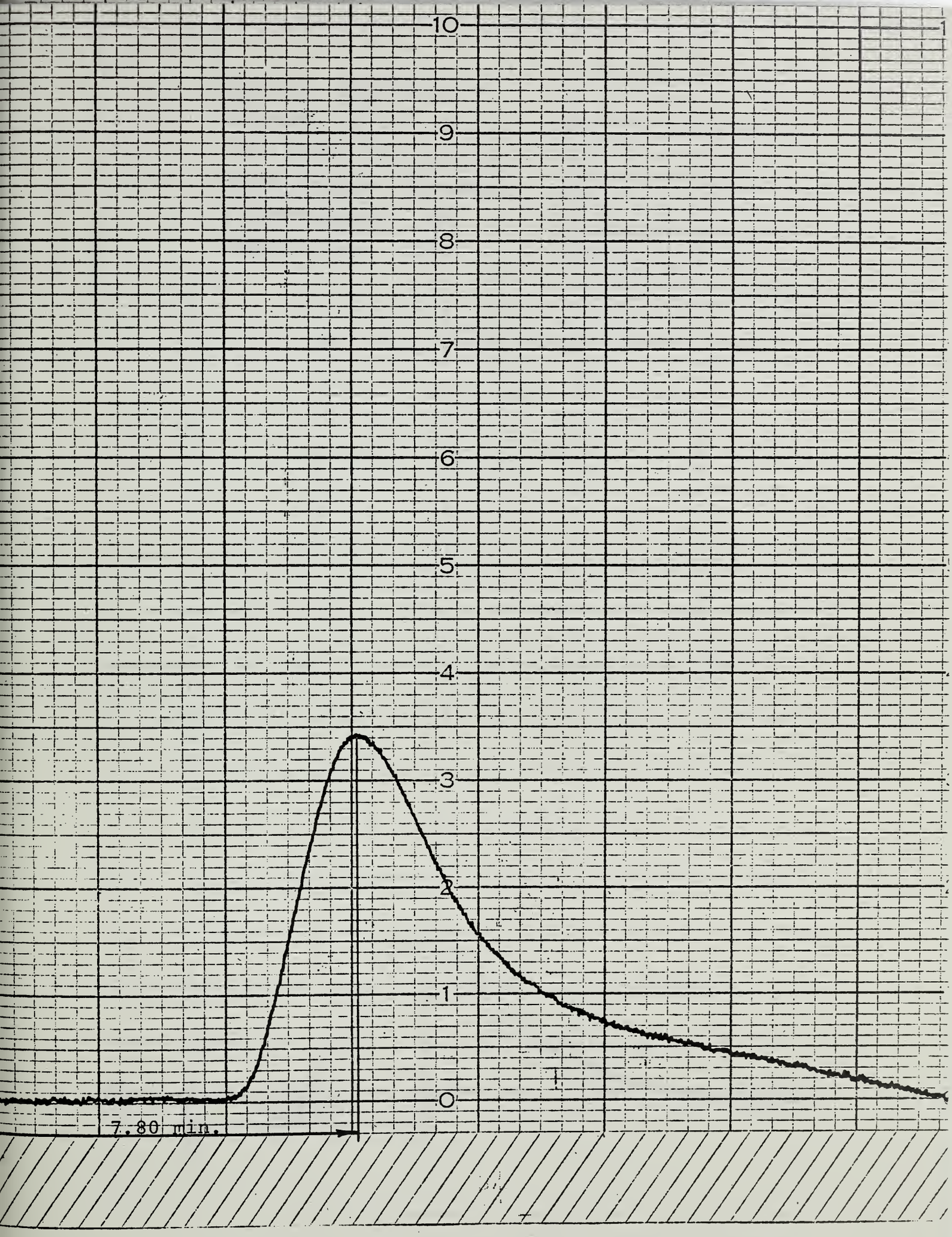

Figure 15. HPLC chromatogram of Stachyose, at a flow rate of 0.5 ml/min.

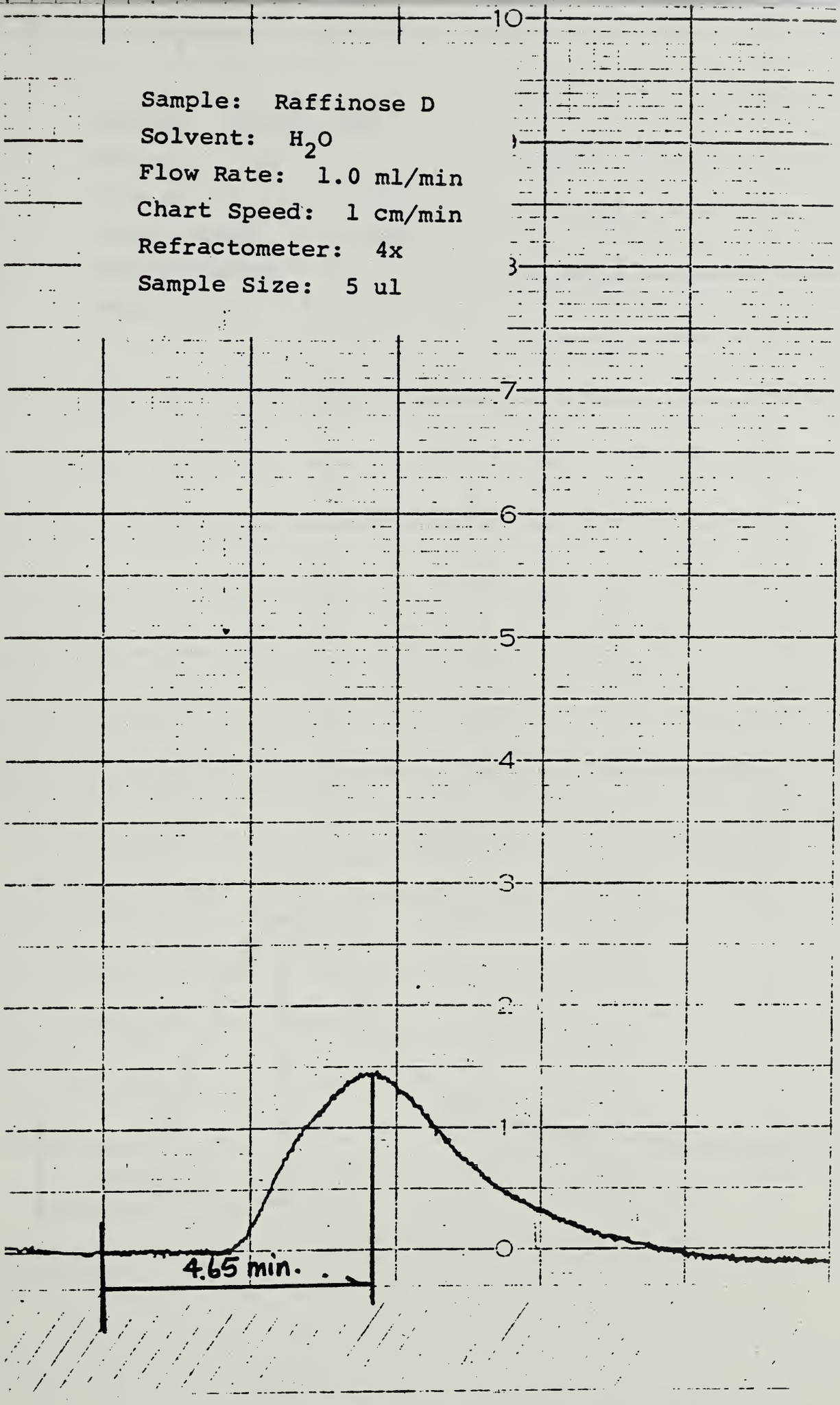

Figure 16. HPLC chromatogram of Raffinose, from previous report.

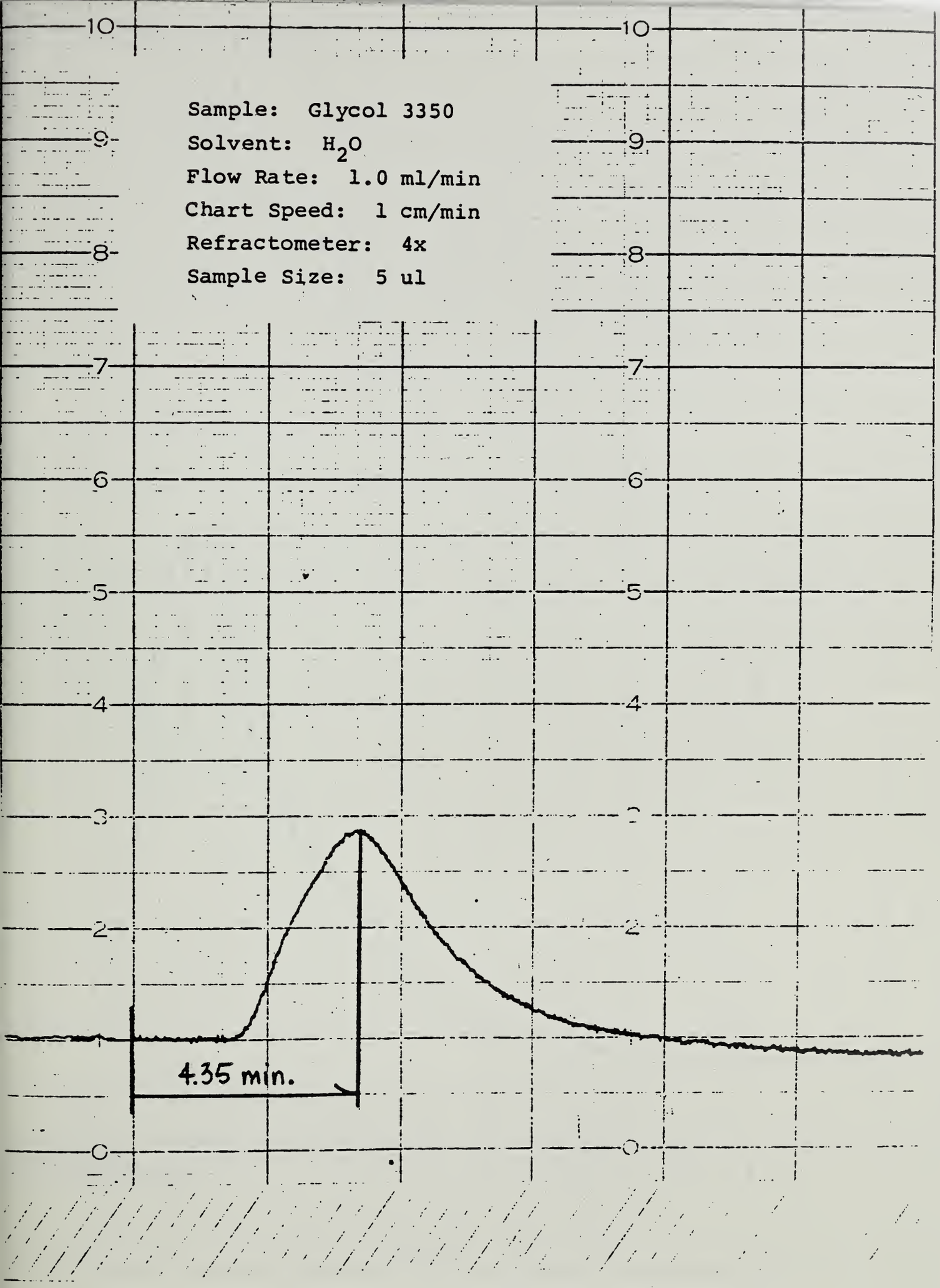

Figure 17. HPLC chromatogram of 3350 PEG, from previous report.

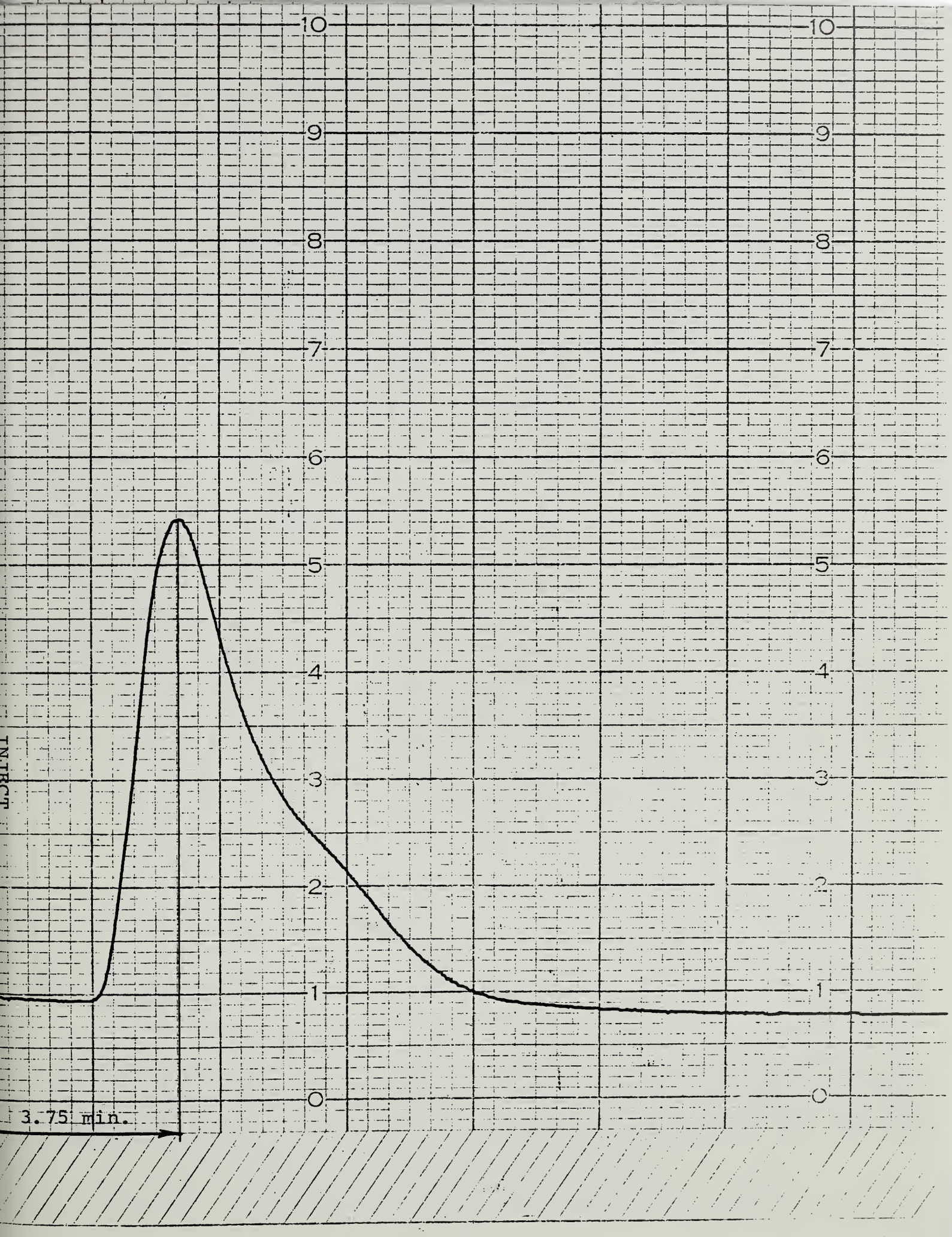

Figure 18. HPLC chromatogram of Methanol.

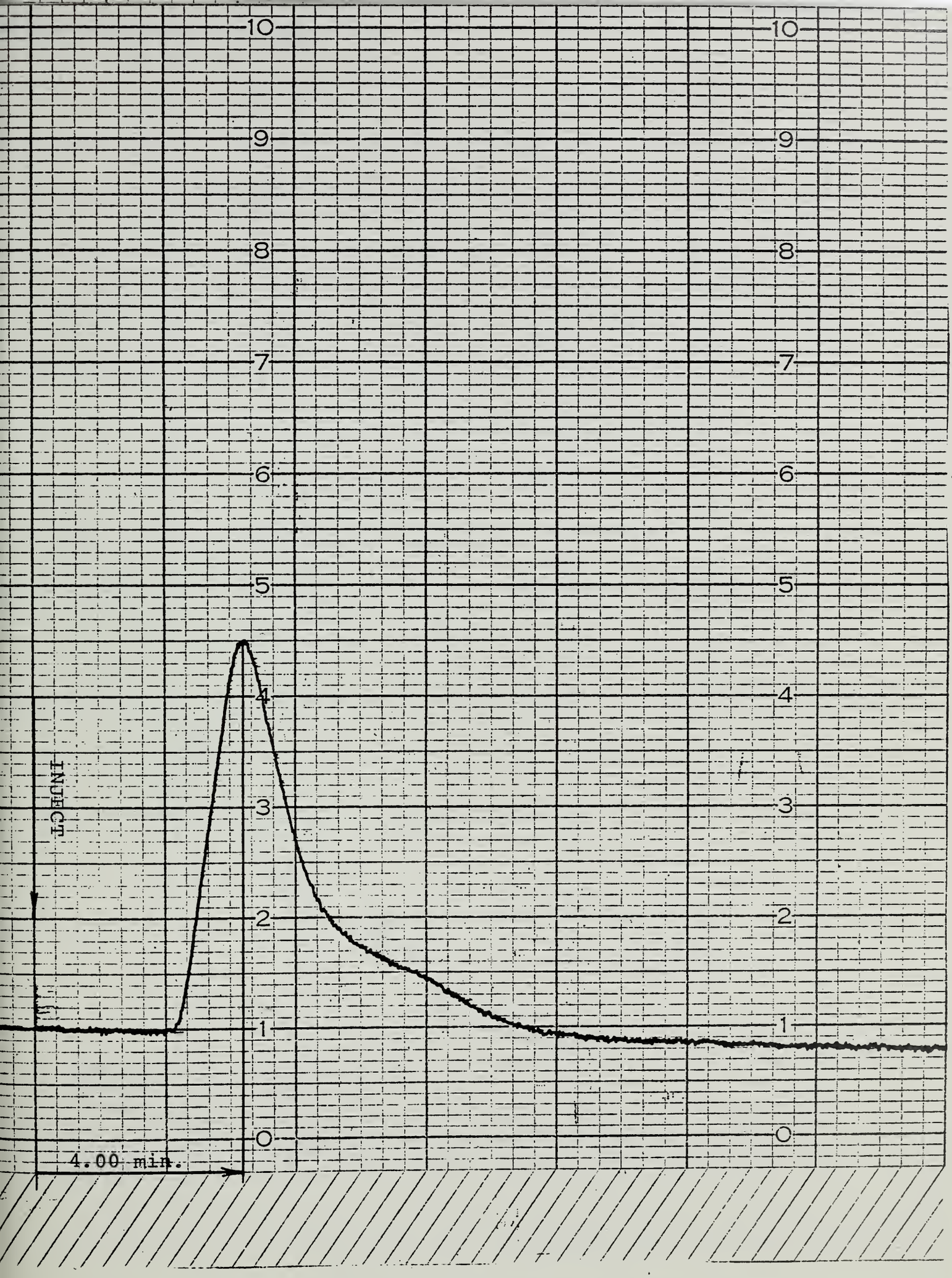

Figure 19. HPLC chromatogram of a 1:1 mixture of 6000 and 200 Polyethylene Glycol.

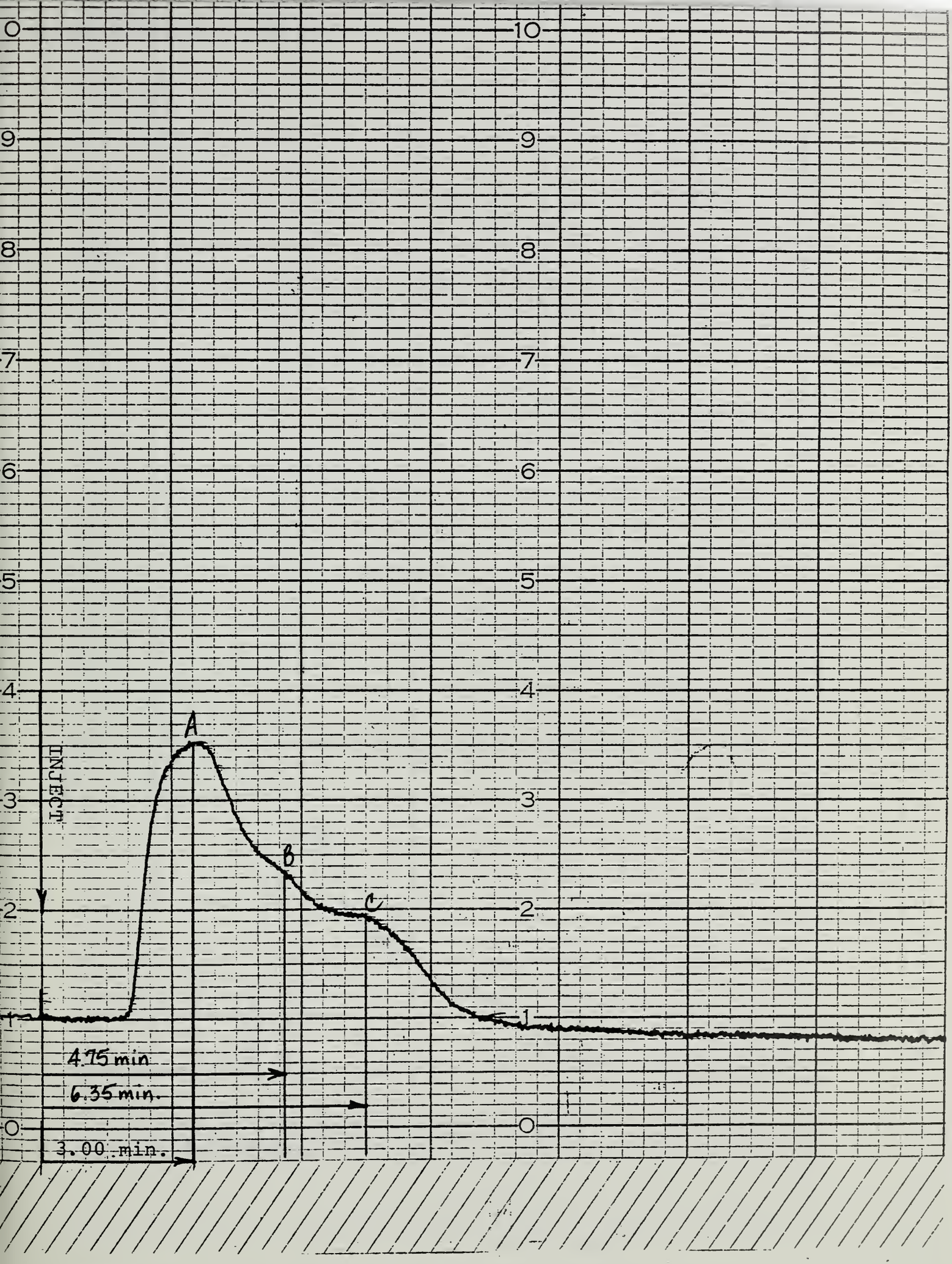

Figure 20. HPLC chromatogram of 200 PEG, under increased column pressure.

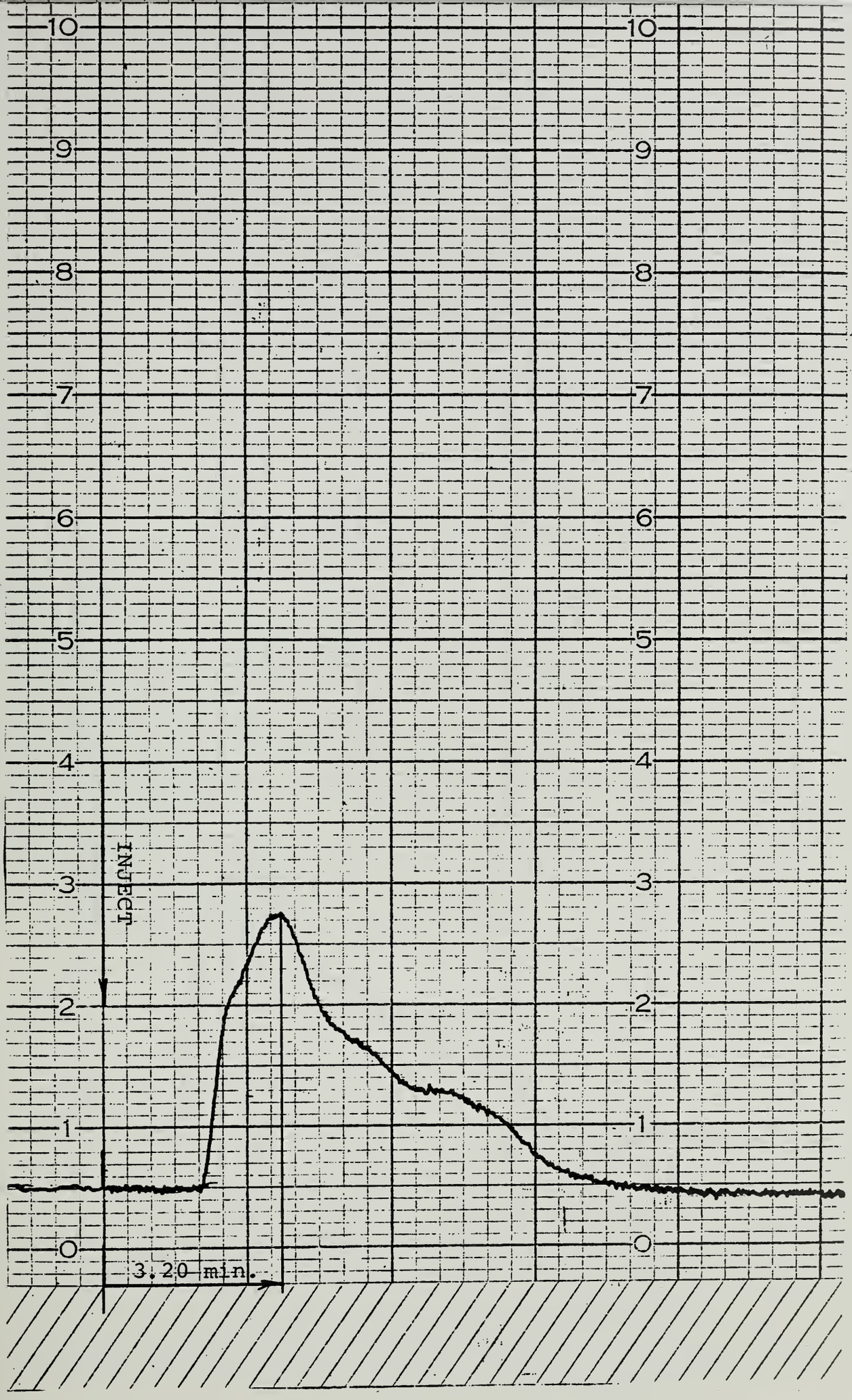

Figure 21. HPLC chromatogram of 6000 PEG, under increased column pressure.

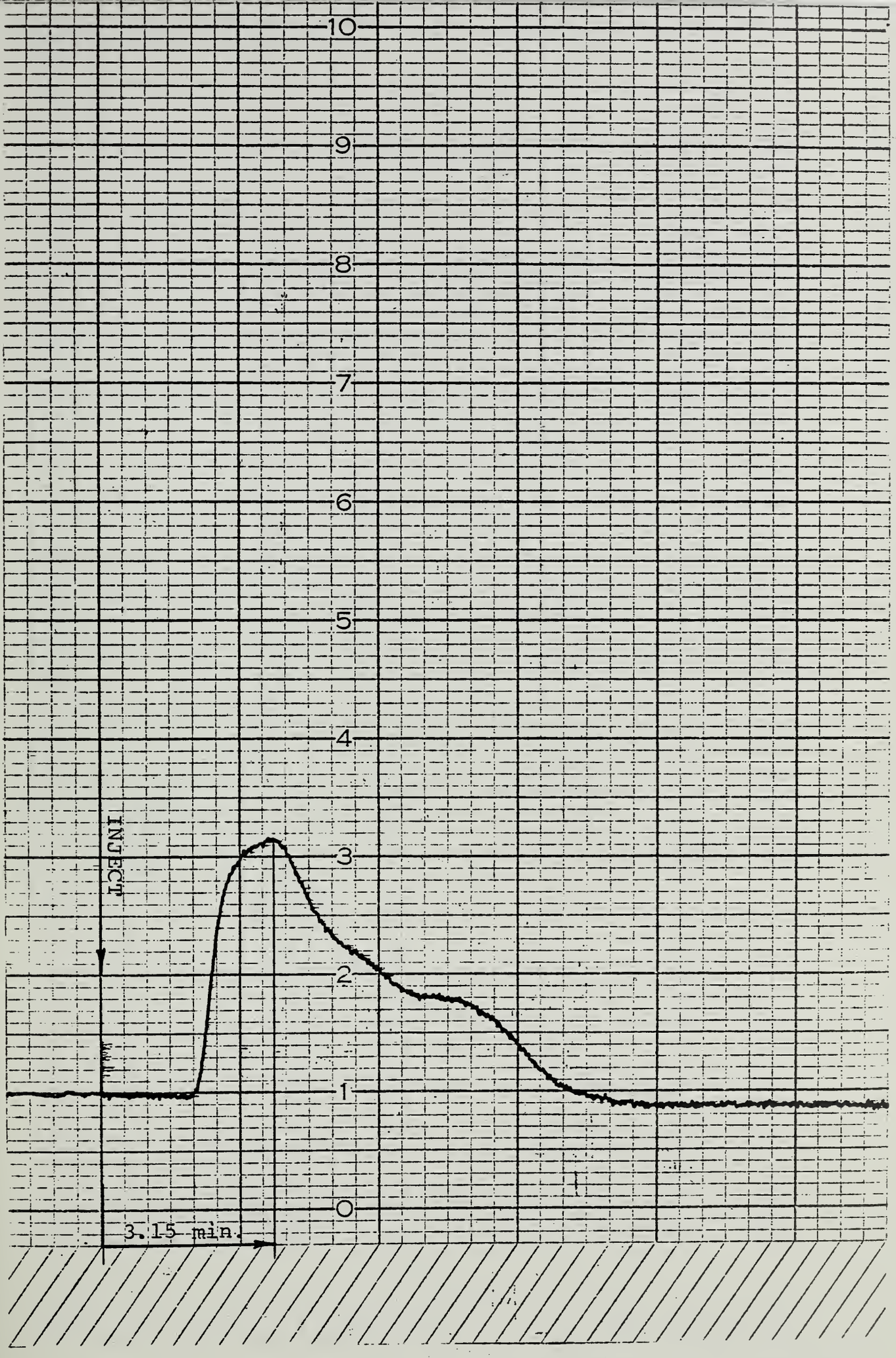

Figure 22. HPLC chromatogram of Glucose, under increased column pressure.

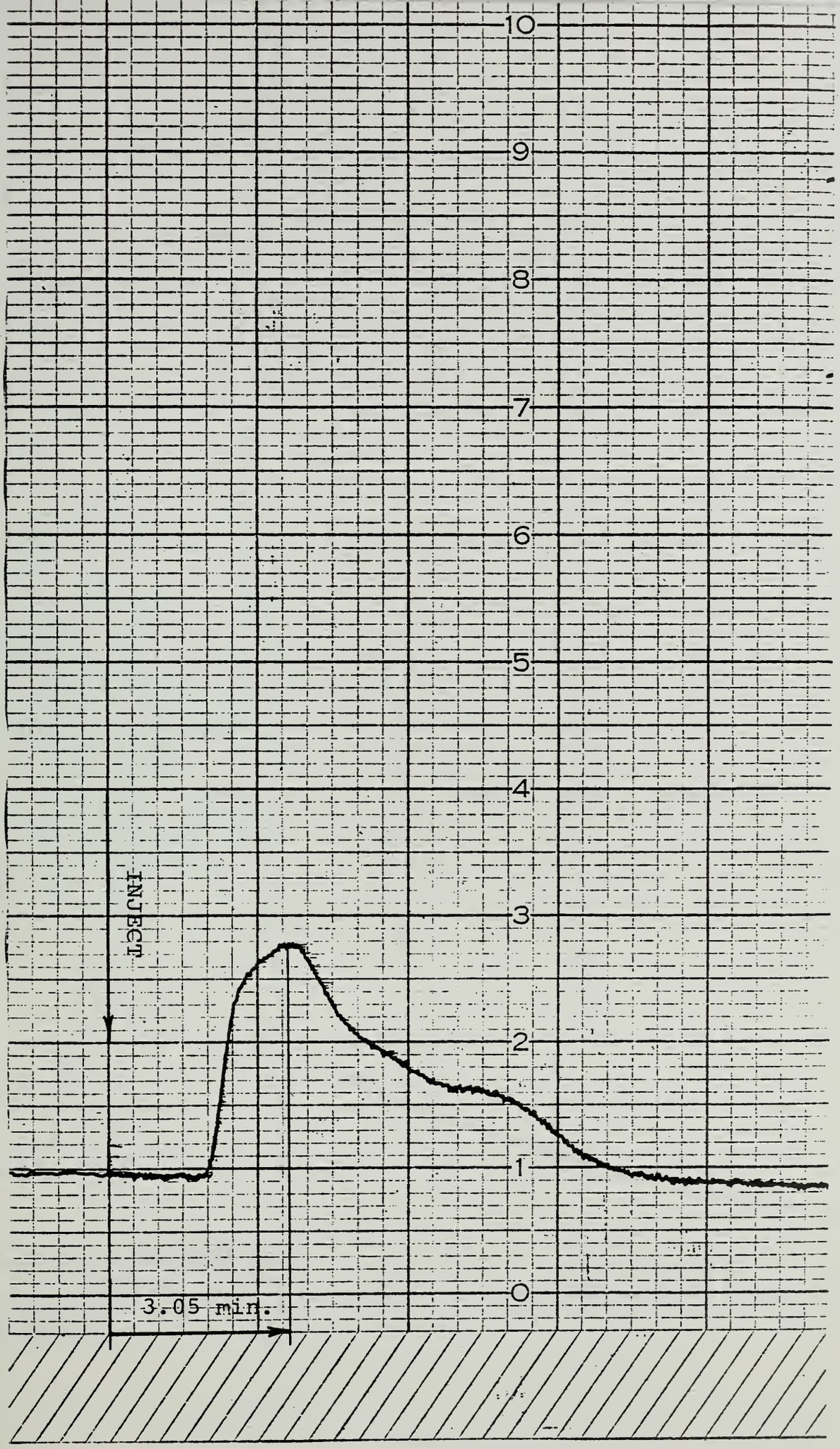

Figure 23. HPLC chromatogram of Raffinose, under increased column pressure.

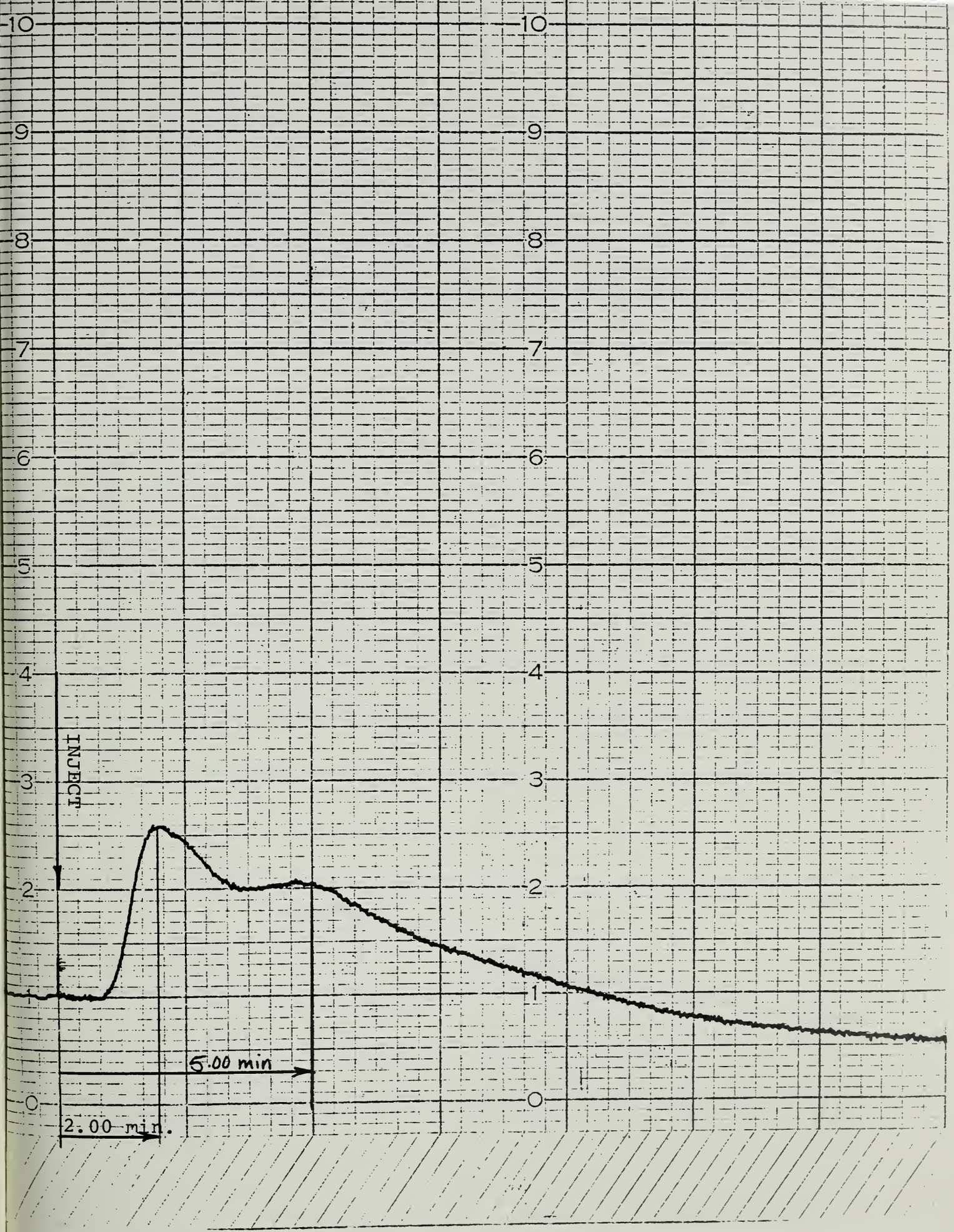

Figure 24. HPLC chromatogram of 200 PEG under reduced column pressure.

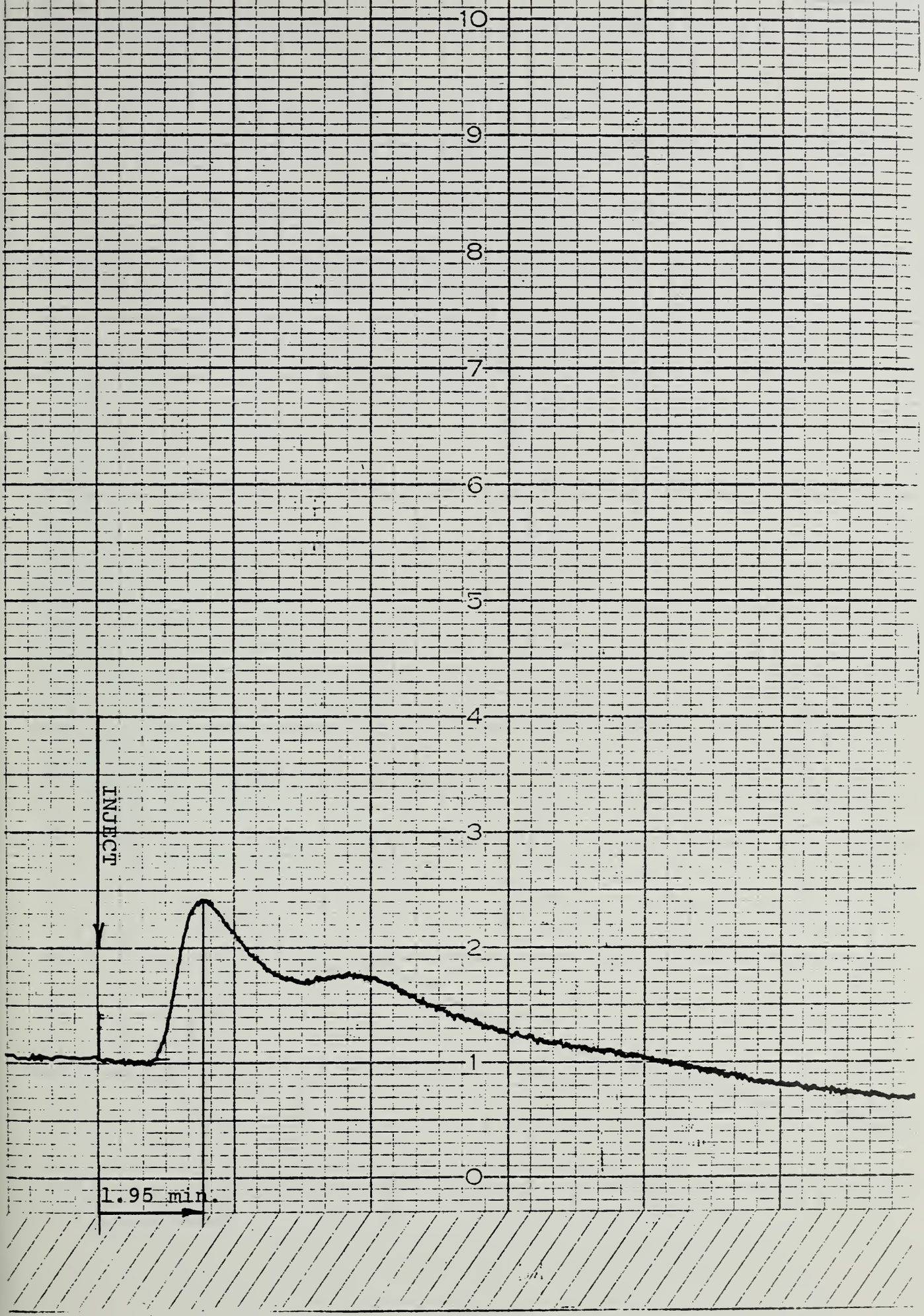

Figure 25. HPLC chromatogram of 6000 PEG under reduced column pressure.

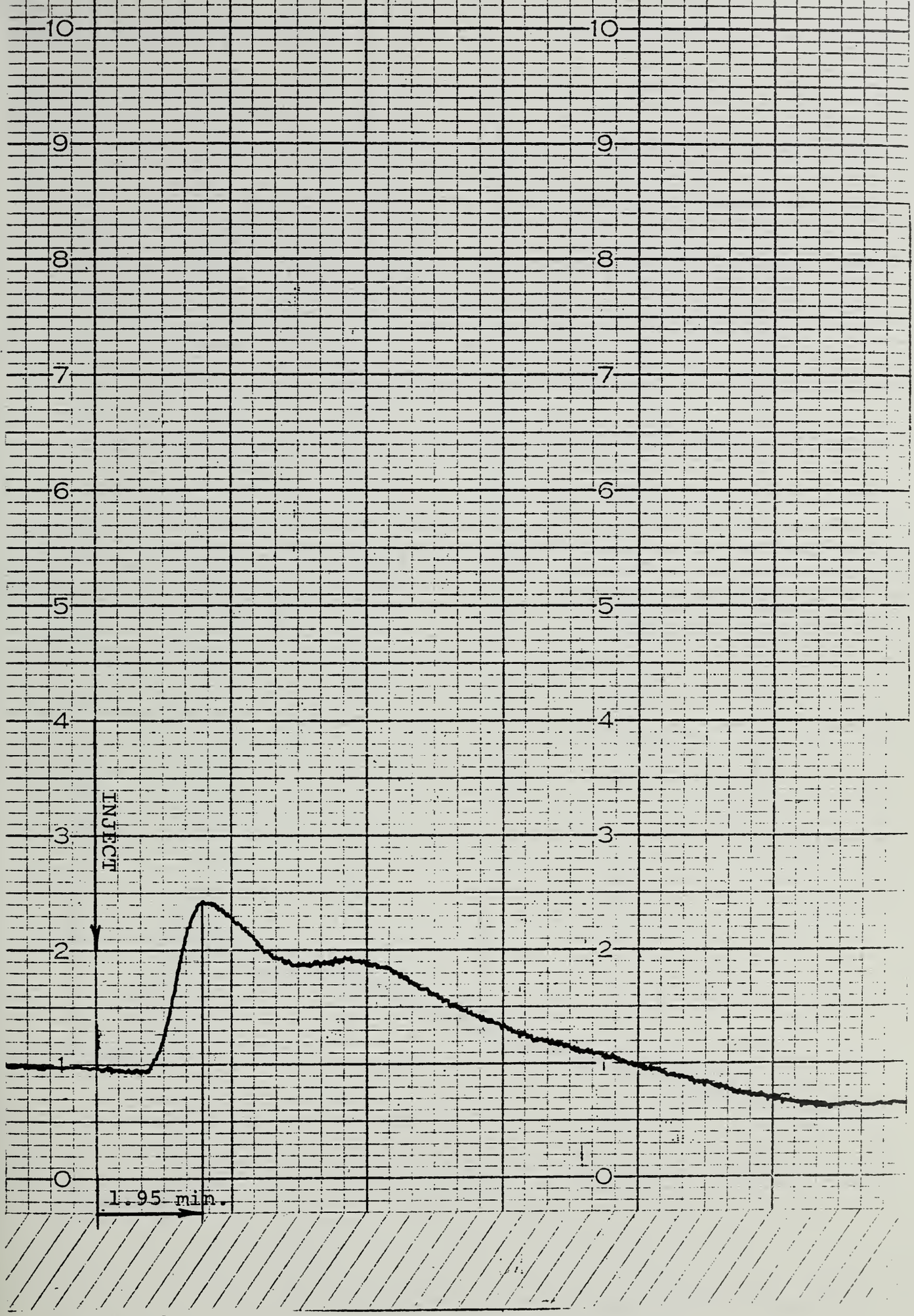

Figure 26. HPLC chromatogram of Glucose, under reduced column pressure.

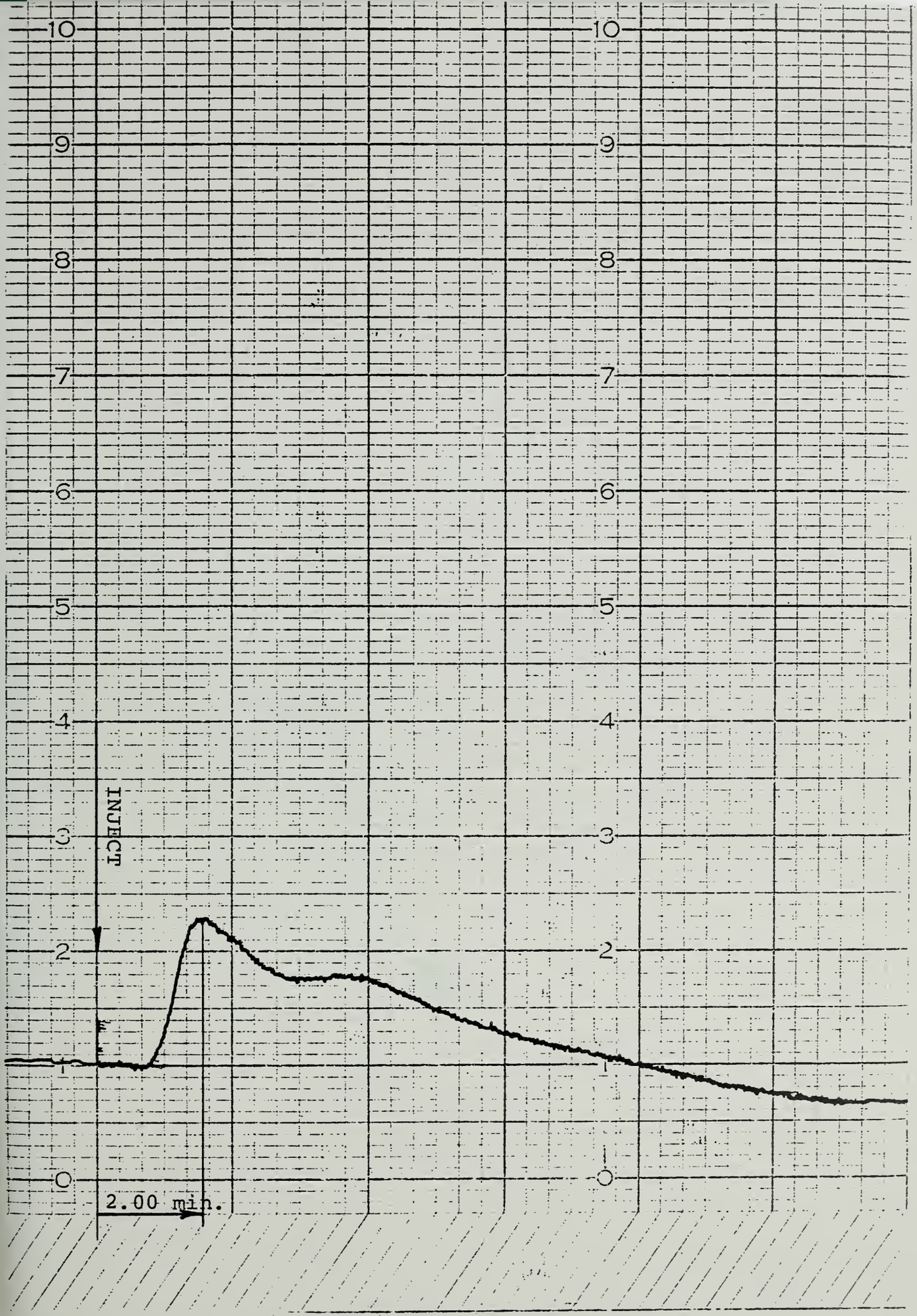

Figure 27. HPLC chromatogram of Raffinose, under reduced column pressure.