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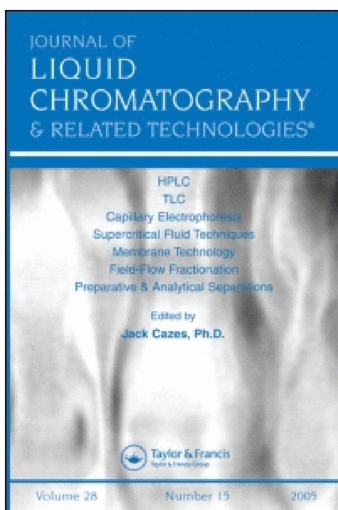

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An Improved Method for the Purification of Hepatic Proliferation Inhibitor by Anion-Exchange HPLC

J. B. McMahon[a]; P. T. Iype[a]
[a] NCI-Frederick Cancer Research Facility, Frederick, MD

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AN IMPROVED METHOD FOR THE PURIFICATION OF HEPATIC PROLIFERATION INHIBITOR BY ANION-EXCHANGE HPLC

J.B. McMahon and P.T. Iype

NCI-Frederick Cancer Research Facility
Frederick, MD 21701

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ABSTRACT

An improved method for the purification of hepatic proliferation inhibitor from rat liver by means of anion-exchange HPLC has been developed. The inhibitor can be purified on an anion exchange HPLC column by using a linear sodium phosphate gradient. The HPLC method allows repeated use of one column and is both rapid and reproducible. The hepatic proliferation inhibitor isolated by this method retains all of its biological activity and is homogeneous as revealed by reverse-phase HPLC.

INTRODUCTION

The search for endogenous factors which control the proliferation of mammalian cells has been the subject of extensive investigation for decades (1,2,3). However, the lack of purity of most of the preparations used has made it difficult to delineate their actual biological role. We have recently purified a rat liver protein which inhibits the proliferation of nonmalignant rat liver epithelial cells (4). Using DEAE-cellulose chromatography we have shown that this hepatic proliferation inhibitor (HPI) can be effectively purified. Since this procedure is both tedious and

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time consuming, a simple and fast method was developed for the isolation of HPI using anion-exchange HPLC which offers a degree of resolution similar to that of electrophoretic methods (5).

MATERIAL AND METHODS

The starting material used in this study was prepared as described earlier (4,6). Prior to anion-exchange HPLC, the material was dialyzed first against 0.05% acetic acid for 12 hrs and then against 0.005M sodium phosphate, pH 6.0 for an additional 12 hrs. Anion-exchange HPLC separations were performed on a 250 x 4.1 mm ID SynChropak AX 300 column (SynChrom, Inc., Linden, IN). A Waters high-pressure liquid chromatography system which included a Model 450 variable wave length detector (Schoeffel Instrument Corp., Westwood, NJ); a M-660 solvent programmer; a U6K sample injector and two M-6000 solvent delivery pumps was used. For anion-exchange HPLC 0.5 ml samples consisting of 10 mg of protein in 0.005M sodium phosphate, pH 6.0 were injected and were eluted isocratically for 10 min in the same buffer at a flow rate of 0.5 ml per min. At this time, the samples were eluted with a 20 min linear gradient to 0.092 M sodium phosphate buffer, pH6.0, followed by isocratic elution using the same buffer. The uv absorbance of the column effluent was monitored at 260 nm. A reequilibration time for repeat injections of 20 min was used routinely. Reverse-phase HPLC separations were performed on a 250 x 4.6 mm ID Altex Ultrashere ODS column (Alltech Associates, Arlington Heights, IL) according to the method of Henderson *et al*.(7).

RESULTS

Fig. 1 shows the profile of the starting material chromatographed by anion-exchange HPLC. The technique resolved the sample into a number of components. The major portion of material eluted during the 10 min isocratic step. Application of the

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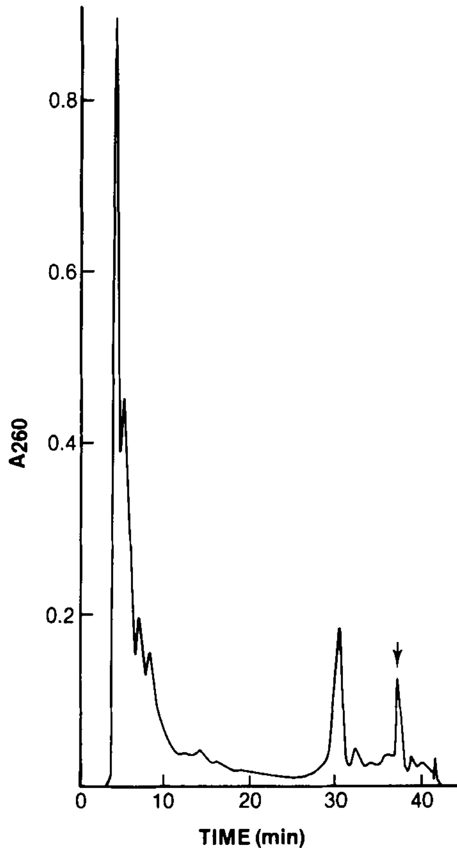

Fig. 1 Anion-exchange HPLC of starting material from rat liver. Sample volume was 0.5 ml consisting of 10 mg of protein in 0.005 M sodium phosphate, pH 6.0. The column was eluted isocratically for 10 min in the same buffer followed by elution with a linear 20 min gradient to 0.092 M sodium phosphate, pH 6.0 at a flow rate of 0.5 ml/min and isocratic elution with the final buffer. Arrow indicates the active inhibitory peak.

linear sodium phosphate gradient resolved the retained material into several peaks. The inhibitory activity resided in the peak which eluted 37 min after sample injection. When this peak fraction was collected and rechromatographed under identical conditions a single peak again eluted 37 min after sample application (Fig. 2). The active peak fraction was analyzed by reverse-

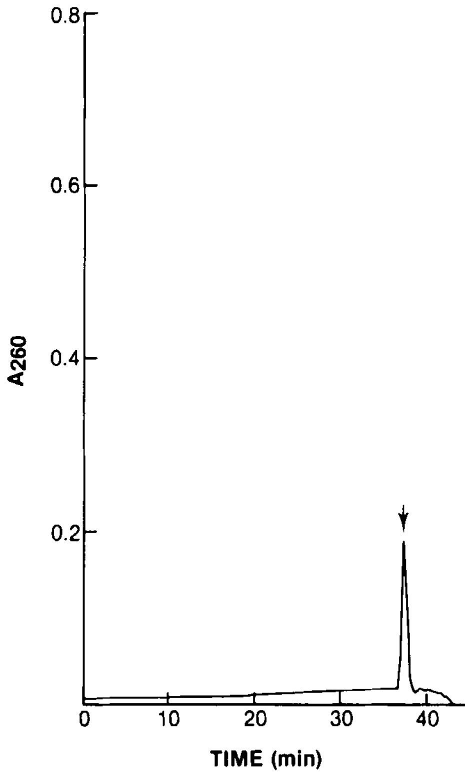

Fig. 2 Anion-exchange HPLC of HPI purified from the starting material by anion-exchange HPLC. Sample volume was 0.5 ml consisting of approximately 100 μg of protein. Chromatographic conditions identical to Figure 1.

phase HPLC and the purified inhibitor eluted from the column as a single sharp peak at 8% acetonitrile (Fig. 3).

DISCUSSION

In a previous report (4) we showed that HPI can be purified to apparent homogeneity by DEAE-cellulose chromatography. Although this method proved to be satisfactory, it was very time consuming, since the column had to be washed extensively before HPI could be effectively isolated by salt elution. In contrast, this is

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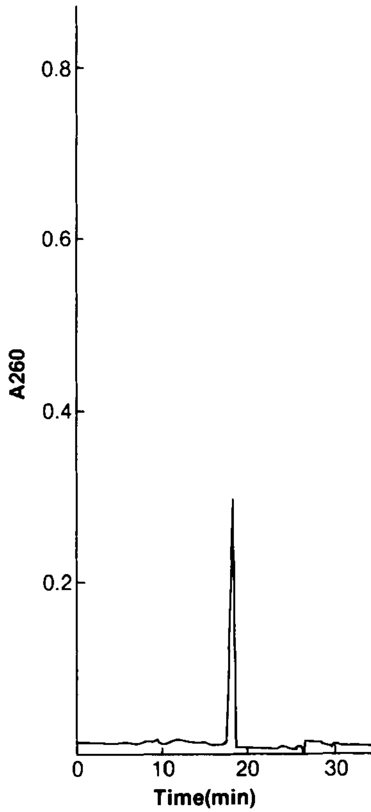

Fig. 3 Reverse-phase HPLC of HPI purified by anion-exchange HPLC. Sample volume was 0.5 ml consisting of approximately 100 μg of protein. Solvent A: 0.05% trifluoroacetic acid in water; solvent B: 0.05% trifluoroacetic acid in acetonitrile; Gradient 0% solvent B to 20% solvent B over 30 min at a flow rate of 1 ml per min.

not the case with anion-exchange HPLC where most of the contaminated material is removed from the column in less than 10 min (Fig. 1). The ability to use the HPLC column repeatedly and the short reequilibration time (20 min) are also obvious advantages. This new procedure, while saving considerable time, in no way altered the properties of the purified HPI, since its chromato-

graphic profile (Fig. 1) and biological activity (data not shown) were similar to that obtained with the HPI purified with DEAE-cellulose (4). Furthermore, the reverse-phase HPLC profile of the material isolated by anion-exchange HPLC (Fig. 3) is in good agreement with that previously published for the HPI isolated by DEAE-cellulose chromatography (4).

This demonstrates that the purification of HPI can be achieved readily with anion-exchange HPLC. This technicque is useful, not only as a preparative method where relatively large amounts (10 mg) of the starting material can be effectively separated on a column of the size and type described, but also can be modified for use as an analytical method.

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