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Note

Preparative-scale high-performance liquid chromatography of ferricrocin, a microbial product*

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The advantages of high-performance liquid chromatography (HPLC) have been demonstrated in a large number of publications. Of interest is not only its analytical application but also the transformation of the analytical results to a preparative scale for isolation of substances of high purity. Such substances are needed as standards in analytical HPLC or for the elucidation of structure.

The preparative HPLC reported here was not carried out on an analytical column having a small diameter followed by collection of the fractionated eluate, but in large-diameter columns, as has been reported by other workers[1–4].

EXPERIMENTAL

Apparatus

A simple isocratic preparative HPLC system (Fig. 1) was constructed from a Haskel Model 26740 air-driven pump (Haskel, Burbank, CA, U.S.A.), a Rheodyne Model 7120 sample injector (Rheodyne, Berkeley, CA, U.S.A.) containing 100-μl and 2-ml sample loops and a Knauer Model 8100 spectrophotometric detector (Knauer, Berlin, G.F.R.), equipped with a preparative cell (pathway 2 mm).

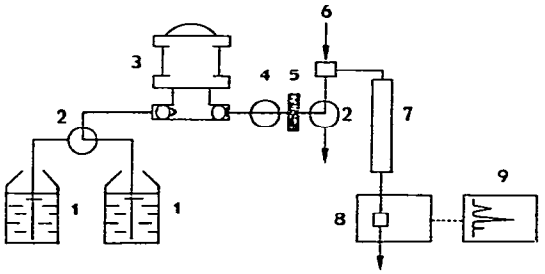

Fig. 1. Schematic set-up of a preparative high-performance liquid chromatograph. 1 = Solvent reservoir; 2 = three-way valve; 3 = pump; 4 = shut-off valve; 5 = solvent filter; 6 = injection valve; 7 = column; 8 = detector; 9 = recorder.

* No. 194 in the series Metabolic products of microorganisms.

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The reversed-phase columns packed with Merck LiChrosorb RP-8 (7 μm) (250 × 16 or 4.6 mm I.D.) were obtained from Knauer.

The Whitey valves, Nupro solvent filter and Swagelok fittings were purchased from Kontron (München, G.F.R.).

Reagents

Double distilled water was used. Acetonitrile GR was obtained from E. Merck (Darmstadt, G.F.R.).

Chromatographic conditions

Stationary phase: LiChrosorb RP-8 (7 μm). Mobile phase: water–acetonitrile (9:1); flow-velocity 12 cm/min. Column temperature: ambient. Detector: wavelength 270 nm; sensitivity, 2.0 absorbance units full scale.

Ferricrocin isolation

Ferricrocin, a metabolic product from *Aspergillus viridi-nutans* (Fig. 2), was isolated from the fermentation broth by adsorbing the culture filtrate on Amberlite XAD-2, as described recently[5]. The product was lyophilized and known amounts were dissolved in water and injected onto the HPLC column.

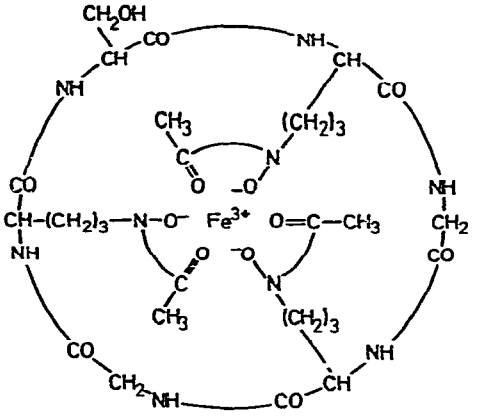

Fig. 2. Structure of ferricrocin.

RESULTS AND DISCUSSION

In an initial investigation, the efficiency of an analytical column was compared with the preparative column. When using the same particle size (7 μm), and the same sample weight and flow-rate relating to the column diameter, a reduction of about 10% was observed in the efficiency of the preparative column in comparison with the analytical column (Table I). This reduction is in contrast to results of Wolf[6] and Wehrli[7], who found a higher efficiency for the preparative column.

When the efficiency is determined by use of an ideal testing substance, such as naphthalene, the plate height will be reduced by a factor of 2. Ferricrocin is not an

TABLE I
EFFICIENCY OF THE ANALYTICAL AND PREPARATIVE COLUMNS

Column diameter (mm)	*Sample weight (mg)*	*Capacity factor, k'*	*Plate height, H (mm)*
4.6	1.0	3.8	0.078
16.0	12.5	4.0	0.087

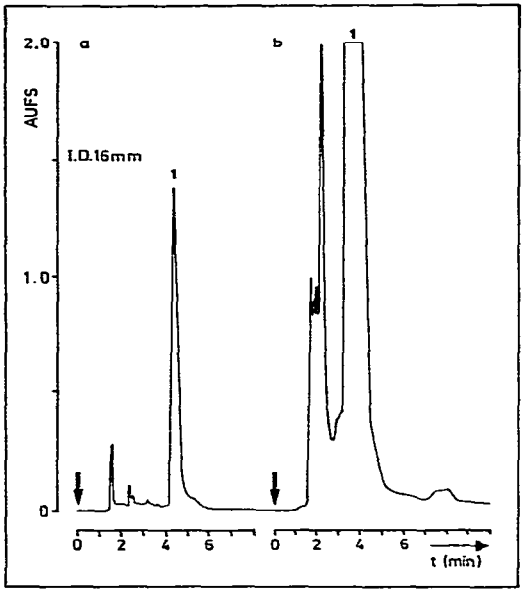

Fig. 3. Effect of column overload: a, 100 mg ferricrocin; b, 1 g ferricrocin. 1 = Ferricrocin.

ideal substance for determination of column efficiency because of its low molar extinction coefficient ($\varepsilon_{270\,nm} = 840\ l \cdot mol^{-1} \cdot cm^{-1}$) and polar character.

In a second investigation the loading limit of the preparative column was determined. Column overload in preparative chromatography is defined as a reduction in the k' values by 10% or more from the values for analytical separations[3]. This effect is shown in Fig. 3, when loading the column with 100 mg and 1 g, respectively. Table II demonstrates the dramatic reduction in the efficiency and increase in the plate height when column overload occurs. The k' value was reduced by 20% and the

TABLE II
COLUMN OVERLOAD

Sample weight (g)	*Capacity factor, k'*	*Plate height, H (mm)*
0.1	3.2	0.21
1.0	2.6	1.84

efficiency to 40% when a eight-fold sample weight (100 mg) was injected. When an 80-fold sample weight (1 g) was injected, the k' value was reduced by 35% and the efficiency to 5%.

In spite of a drastic reduction in the efficiency on column overloading, preparative HPLC is an ideal method for isolation of substances of high purity. Its advantage is founded on the short separation time which is derived from a high sample throughput and a high sample purity. In addition, the small peak volume is advantageous for the isolation of the separated substance from the eluent[2].

The capacity limit in the separation problem described above was reached with a sample weight of 20 mg per g stationary phase.

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