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## Note

# Determination of fructan oligomers of degree of polymerization 2–30 by high-performance liquid chromatography

W. PRAZNIK* and R. H. F. BECK
*Universität für Bodenkultur, Institut für Chemie, Gregor Mendelstrasse 33, A-1180 Vienna (Austria)*
and
E. NITSCH
*Laevosan Gesellschaft Linz, Estermannstrasse 17, A-4020 Linz (Austria)*
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The separation of oligosaccharides is an important task for biochemists and polymer and natural product chemists. Paper[1–3], thin-layer[4–6] and column chromatography[1,7,8] (Celite carbon, charcoal) were first used and gave a separation of oligosaccharides up to a chain length of 20 monomeric units[3], but with good resolution only up to 10 monomeric units. The quantification of the oligosaccharide fraction was difficult and time consuming.

Since then, the application of high-performance liquid chromatographic (HPLC) methods to the analysis of oligosaccharides has been widely studied[9–19]. The use of cation-exchange resins, mainly used in the $Ca^{2+}$ (ref. 9) or $Ag^{+}$ (ref. 10) form with an aqueous eluent, led to the separation of oligomers up to a chain length of 8. Other workers used commercially available weak ion-exchange resins[11–13] ($NH_2$-silica columns) with an organic eluent. These columns with chemically bonded phase separated the oligomers up to a degree of polymerization (D.P.) of 7 (ref. 14), 10 (ref. 15) or 15 (ref. 16). This type of column has two main disadvantages: a high price and a high "mortality rate". The separation of carbohydrates by impregnating silica columns with amines[17–19] is also possible. Monosaccharides and low-molecular-weight oligosaccharides, and later also malto-oligosaccharides, showed good resolution up to a D.P. of 20.

In this paper we describe an HPLC system for the separation of fructan polymers with a D.P. of up to 30 using an amine-modified silica column.

## EXPERIMENTAL

### *HPLC apparatus, column and operating conditions*

The liquid chromatographic system was constructed from different commercially available components. The eluent was degassed by use of an ultrasonic bath (Bransonic, Branson, The Netherlands) and filtered through a 0.5-μm FH membrane filter (Millipore, Bedford, MA, U.S.A.). A Model 6000 pump (Waters Assoc., Milford, MA, U.S.A.) delivered the solvent to the 40 × 4.0 mm I.D. pre-column (Spherisorb, 10 μm; Knauer, Bad Homburg, F.R.G.) and to a heated [35°C, maintained

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with an FJ water-bath (Haake, Berlin, F.R.G.)] 250 × 4.0 mm I.D. column (Spherisorb, 5 μm, Knauer). Samples of 20 μl of a 10% aqueous solution, injected into the HPLC system with a Rheodyne 7125 system (Rheodyne, Cotati, CA, U.S.A.), were filtered through a 0.2-μm filter (Sartorius, Göttingen, F.R.G.). An RI detector (Waters Model 401) was used. The chromatograms were recorded on an HP 3380S recorder (Hewlett-Packard, Vienna, Austria).

Amination of columns was carried out overnight with the amination reagent: acetonitrile (Promochem)–distilled water–polyethylene glycol 35,000 (Hoechst, Frankfurt/M, F.R.G.)–putrescin (1,4-diaminobutane) (Merck) (50:50:0.2:0.02). The solvent had the same composition varying only in the putrescin concentration (0.02%).

All solvents were of HPLC grade.

*Preparation of sample*

For the determination of the fructosans a freeze-dried aqueous extract of fresh Jerusalem artichoke (*Helianthus tuberosus*) was used. For the preparation of analytical-reagent grade inulin, Jerusalem artichoke extract was stored in a refrigerator at 4°C for 1 week for crystallization. Recrystallization was carried out twice. Commercial inulin (chicory) was provided by Laevosan (Linz, Austria).

## RESULTS AND DISCUSSION

Fig. 1 shows the carbohydrate composition of Jerusalem artichoke tubers. In

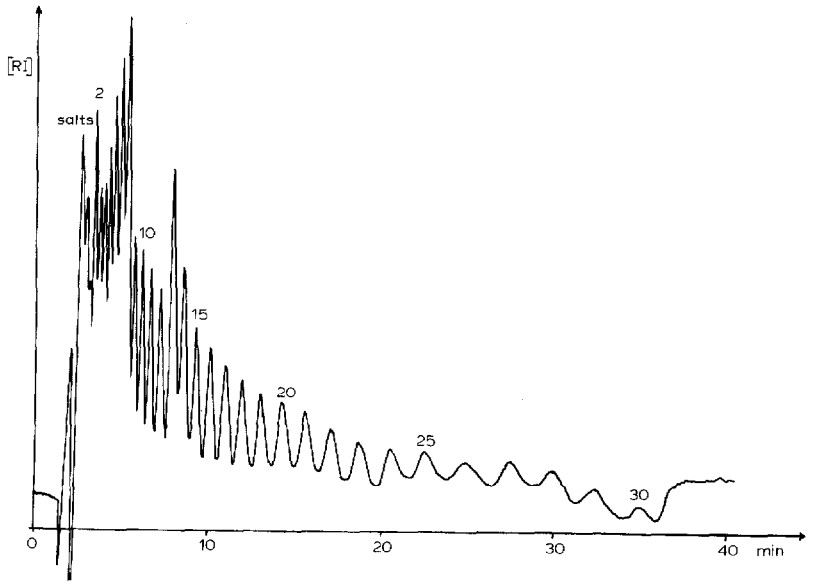


Fig. 1. HPLC separation of native inulin from Jerusalem artichoke. The numbers above the peaks correspond to the degree of polymerization. Volumes of 20 μl of a 10% aqueous sample were injected. Column, Spherisorb, 5 μm, 250 × 4.0 mm I.D.; pre-column, Spherisorb, 10 μm, 40 × 4.0 mm I.D.; temperature, 35°C; solvent, acetonitrile–water–polyethylene glycol–putrescin (50:50:0.2:0.02); flow-rate, 1 ml/min; RI detector, 8 ×; chart speed, 0.5 cm/min.

contrast to commercially available chemically bonded phase $NH_2$-silica columns, the "autoaminifying" column system shows a significantly higher resolution and a better separation of fructo-oligomers. The term "autoaminifying" means that the amount of putrescin bound to silica is kept constant during the whole separation by dissolving the diamine in the eluting solvent. Our HPLC system shows an exact resolution of inulin up to a D.P. of 30.

Fig. 2 shows the separation of a twice recrystallized inulin of Jerusalem artichoke. In contrast to Fig. 1, no low-molecular-weight oligomers appear in this chromatogram because inulin is insoluble in cold water. The distribution of oligomers confirms that only the high-molecular-weight fraction of polymers is involved in the crystallization process.

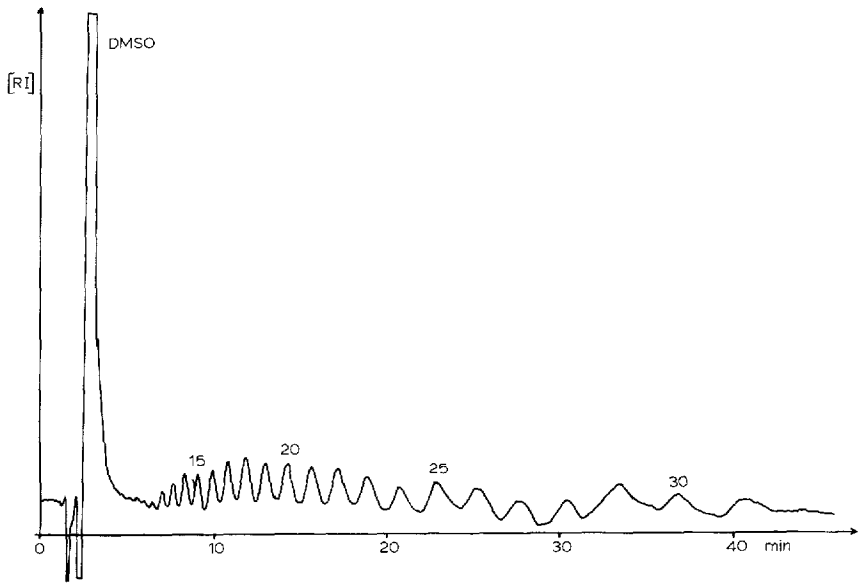


Fig. 2. HPLC separation of recrystallized inulin from Jerusalem artichoke. The numbers above the peaks correspond to the degree of polymerization. Volume of 20 $\mu$l of a 10% solution of inulin in dimethyl sulphoxide (DMSO)–water (90:10) were injected. HPLC conditions as in Fig. 1.

An inulin (Fig. 3) produced commercially shows a different elution profile to the native artichoke extract (Fig. 1) or a highly purified inulin sample (Fig. 2).

The results in Figs. 1–3 demonstrate the capability of HPLC for the investigation of the polymer distributions of inulins of different origins or different states of purification. Further advantages of this method for oligomer separation are the following: (1) the HPLC system used allows the rapid separation of fructans, and qualitative analysis can be carried out easily; (2) the low price of the materials necessary for running the HPLC system compared with $NH_2$-silica columns, *i.e.*, the cheaper filling material and its greater stability and life span[19].

The main difference from other published systems[17–19] is the variation in the eluent and the temperature. The improvement in the separation was obtained by

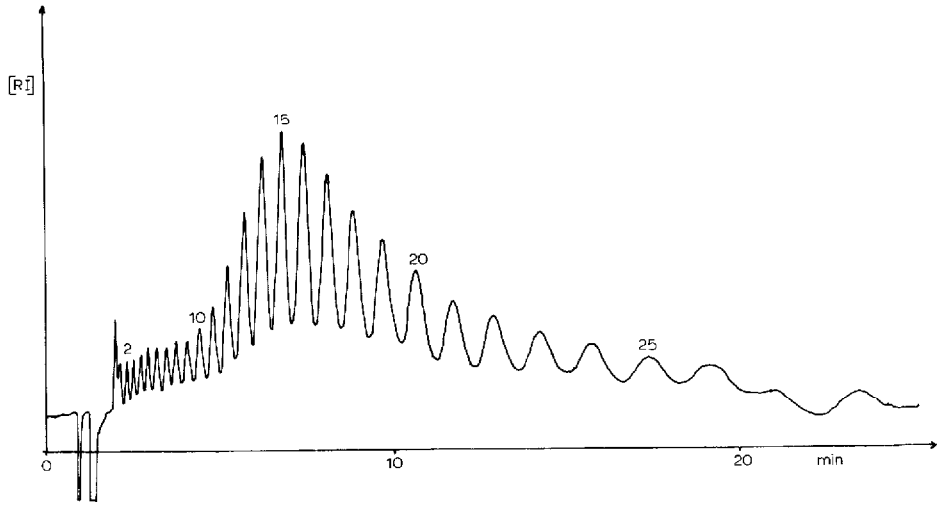


Fig. 3. HPLC separation of commercial inulin from chicory. The numbers above the peaks correspond to the degree of polymerization. Volumes of 20 μl of a 10% aqueous sample were injected. HPLC conditions as in Fig. 1, except flow-rate, 2 ml/min; chart speed, 1 cm/min.

choosing a temperature of 35°C and by adding polyethylene glycol to the eluting system. This temperature gave the optimal resolution. Because of the lower mobility of carbohydrates at lower temperatures, a decrease in temperature led to worse results. The saturation of the silica with putrescin depends on temperature, high temperatures leading to a lower degree of saturation of the silica with the diamine and therefore to a worse separation. The polyethylene glycol seems to influence the binding of the $NH_2$ groups with the OH groups of the saccharides. On adding polyethylene glycol the polarity of the eluting system is lowered, and the $NH_2$–OH binding is weakened.

A separation of oligosaccharides up to a D.P. of 30 is possible, but direct measurement of peak areas and the percentage of inulin compounds is not possible because of the difference in the refractive indices of the single oligomers. For the quantitative analysis of inulins it is necessary to prepare standard oligomers by use of preparative gel permeation chromatography[20] (Bio-Gel P-2, P-4 are mainly used) (Bio-Rad Labs., Richmond, CA, U.S.A.). Using these standards the HPLC system is able to characterize mixtures of fructopolymers quantitatively. It should be applicable to routine analysis in biochemical laboratories that deal with medical (*e.g.*, membrane permeation) research or with the control of biotechnological processes.

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