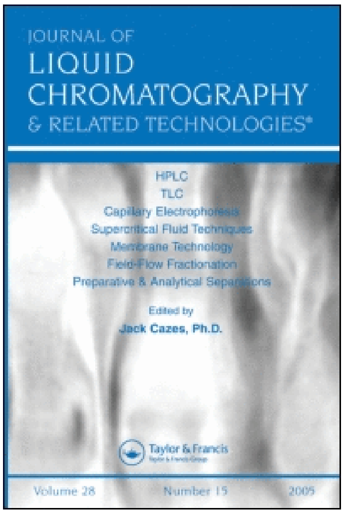


Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Semipreparative High-Pressure Liquid Chromatography of Synthetic Carbohydrates

Robert E. Wingard Jr.[a]; Steve Ng[a]; James A. Dale[a]; Patricia C. Wang[a]
[a] Chemical Synthesis Laboratories Dynapol, Palo Alto, California

To cite this Article Wingard Jr., Robert E. , Ng, Steve , Dale, James A. and Wang, Patricia C.(1978) 'Semipreparative High-Pressure Liquid Chromatography of Synthetic Carbohydrates', Journal of Liquid Chromatography & Related Technologies, 1: 6, 775 — 782

To link to this Article: DOI: 10.1080/01483917808060033

URL: http://dx.doi.org/10.1080/01483917808060033

JOURNAL OF LIQUID CHROMATOGRAPHY, 1(6), 775-782 (1978)

SEMIPREPARATIVE HIGH-PRESSURE LIQUID CHROMATOGRAPHY OF SYNTHETIC CARBOHYDRATES

Robert E. Wingard, Jr., Steve Ng, James A. Dale,
and Patricia C. Wang
Chemical Synthesis Laboratories
Dynapol
Palo Alto, California 94304

ABSTRACT

A rapid and effective method has been developed for the purification of hundred-milligram quantities of water-soluble synthetic carbohydrates. The method utilizes a 30 cm x 7.8 mm i.d. column packed with Waters Associates carbohydrate analysis packing in conjunction with isocratic water-acetonitrile elution and refractive index detection. The generality of this method is illustrated by its application to 13 sucrose derivatives and one derivative each of D-fructose and α,α-trehalose.

INTRODUCTION

As a result of both the increasing recognition of sucrose-related health problems and the continuing uncertainty over the safety of saccharin and many of its alternatives, the last 5 years have witnessed a dramatic expansion in research devoted to finding biologically safe nonnutritive sweeteners (1, 2). A newly emerging area of research in this field involves intensely sweet synthetic carbohydrates (3, 4). The taste panel evaluation (5) of potential sweeteners, although requiring only a few hundred milligrams of an intensely sweet compound, demands that the material being tested be free of off-tasting side products and potentially hazardous residual reactants (1, 6). In our work with synthetic carbohydrate sweeteners, it was found that the complexity of the multifunctional synthetic manipulations, combined with noncrystallinity and insolubility in any but the most polar solvents, render purification to the level necessary for sensory evaluation very difficult.

Numerous classical methods are available for carbohydrate purification (7, 8), but application is often time consuming and results in a considerable loss of material. Furthermore, it has been our experience that the classical techniques, more often than not, provide products of insufficient purity. A number of liquid chromatographic methods exist for carbohydrate analysis, including especially gel permeation (9) and ion exchange (10), but these are not directly suitable for general utilization in the semipreparative mode. Nonaqueous semipreparative high-pressure liquid chromatography (HPLC) has been reported for carbohydrates (11), but its use is limited to a relatively few highly derivatized analogs.

We have developed and report here rapid, efficient, and effective methodology for the semipreparative HPLC isolation and purification of water-soluble synthetic carbohydrates. This method, as demonstrated by its successful application to 15 compounds, appears to be of wide utility.

EXPERIMENTAL

Apparatus

The isocratic HPLC apparatus was constructed from a Model 6000A pump, a Model R-401 differential refractometer (both from Waters Associates, Milford, Mass.), and a Model 7120 syringe loading sample injector (Rheodyne Incorp., Berkeley, Calif.) fitted with a 2-ml injection loop. The recorder employed was a Houston Instruments (Austin, Texas) Omniscribe operated at 10 mv sensitivity and a chart speed of 0.8 in/min.

Semipreparative separations were conducted with a 30 cm x 7.8 mm i.d. column custom packed by Waters Associates with their proprietary carbohydrate analysis packing. Analytical HPLC was done with a Waters Associates 30 cm x 3.9 mm i.d. carbohydrate analysis column.

Materials

The solvent mixtures, prepared from Burdick and Jackson (Muskegon, Mich.) high-purity acetonitrile and freshly distilled water, were filtered, degassed (vacuum), and allowed to stand several hours to thermally equilibrate before use. The sugars were prepared, for the most part, by known methods as referenced in Table 1 and 2. The syntheses of previously unreported materials are to be discussed elsewhere.

Generalized Procedure

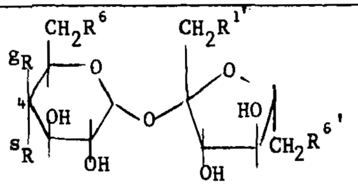
A 0.5 g sample of crude carbohydrate was dissolved in methanol to approximately 40% concentration and the solution filtered through a 0.6 µm poly(vinyl chloride) filter (Millipore Corp., Bedford, Mass.). The solution was examined on the analytical column (5 µl of solution, detector attenuation 16X) at a flow of 2.0 ml/min and the acetonitrile/water ratio adjusted so that the product eluted at 5.0 to 7.5 min. A 3 l. supply of the appropriate solvent mixture was prepared and preparative work then performed with the larger column at 6.0 ml/min utilizing injections of 75 µl and a detector attenuation of 128X. The product was manually collected directly after exit from the detector. Upon completion of each collection, the column was flushed by a 2-ml injection of methanol.

RESULTS AND DISCUSSION

The key to the successful semipreparative HPLC purification of the synthetic carbohydrates employed in this work (Tables 1 and 2) rested on the use of a 30 cm x 7.8 mm i.d. column custom packed by Waters Associates with their carbohydrate analysis packing. This column proved capable of handling 30-35 mg of carbohydrate (as a highly concentrated solution in methanol) per injection with little noticable loss of resolution relative to a 2-3 mg injection with a 30 cm x 3.9 mm i.d. carbohydrate analysis column.

TABLE 1

Sucrose Derivatives Purified by Semipreparative HPLC



Cpd	Substitution Pattern 1'	4^s	4^g	6	6'	Solvent Mix CH_3CN (%)	H_2O (%)	Synthesis Reference
	METHYL ETHERS							
1	OCH_3	OH	H	OCH_3	OCH_3	92.5	7.5	12
2	OH	OH	H	OCH_3	OCH_3	85.0	15.0	12
3	OCH_3	OCH_3	H	OH	OCH_3	87.5	12.5	12
4	OCH_3	OH	H	OH	OCH_3	85.0	15.0	12
5	OH	OH	H	OH	OCH_3	80.0	20.0	12
	CHLORODEOXY DERIVATIVES							
6	OH	OH	H	Cl	Cl	87.5	12.5	13
7	Cl	OH	H	Cl	Cl	87.5	12.5	14,15
8	Cl	H	Cl	OH	Cl	92.5	7.5	15,16
9	Cl	OH	H	OH	Cl	88.5	12.5	15
10	Cl	Cl	H	OH	Cl	95.0	5.0	4
11	OH	H	Cl	Cl	OH	90.0	10.0	4
	DEOXY DERIVATIVES							
12	H	H	H	OH	H	85.0	15.0	4
13	H	OH	H	OH	H	85.0	15.0	4

Differential refractometry was found more suitable for detection than low-wavelength UV. This was the result of the fact that minor UV-active reaction impurities and traces of residual organic

Table 2

Additional Carbohydrate Derivatives Purified

Compound	Solvent Mix CH_3CN (%)	 H_2O (%)	Synthesis Reference
1,6-Dichloro-1,6-dideoxy-D-fructose	92.5	7.5	4
6,6'-Dideoxy-6,6'-difluoro-α,α-trehalose	92.5	7.5	17

solvents tended to obscure the products at the wavelengths necessary for carbohydrate detection. Because of the complexity of some of the reaction mixtures it was found advantageous to collect the desired peak directly after exit from the detector (i.e., no excessive length of tubing employed) and thereby minimize inadvertant contamination.

Acetonitrile-water mixtures were determined to be the best elution solvent. Retention and resolution were, however, found extremely sensitive to the volume of injection solvent. For this reason the carbohydrates were injected as highly concentrated solutions in methanol or occasionally methanol-water (1:1). Methanol was selected for this purpose primarily because of its high solvent power for the carbohydrates under study.

For purposes of resolution and operator efficiency the solvent ratio was adjusted so that the elution time of the product to be isolated was 5.0-7.5 min. This is demonstrated for the 13 sucrose derivatives in Table 1 where it can be seen that the more polar derivatives required higher water concentrations to elute as desired. The solvent mix was developed with the analytical column at 2.0 ml/min and could, with few exceptions, be transposed directly to the larger column at 6.0 ml/min.

By utilizing short retention times and a brief (2-ml methanol injection) column flush after each collection (to remove polar components), it was found that only 10-20 min were required per

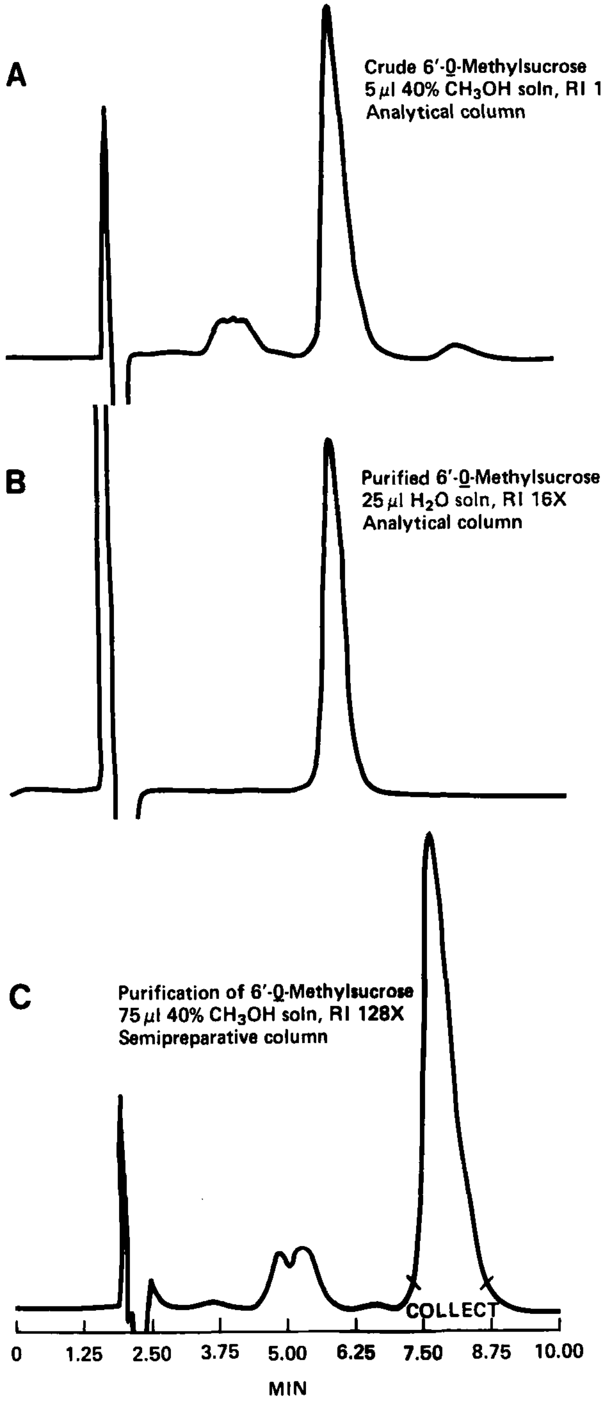


Figure 1. HPLC chromatograms of 6'-O-methylsucrose with 20% H_2O in CH_3CN. Chromatograms A and B (analytical column) show the material before and after purification, respectively. Chromatogram C is a 75-μ injection using the semipreparative column.

injection. It thus proved possible to isolate greater than 0.5 g of purified carbohydrate within one days work.

The general utility of this semipreparative method is illustrated by its successful application to synthetic sucrose derivatives (Table 1) ranging in structure from the methyl ethers (OCH_3 in place of OH) to the chlorodeoxy derivatives (Cl for OH) to the two deoxy analogs (H for OH). Additionally, the technique proved effective for a modified trehalose and a monosaccharide (Table 2).

All but two of the compounds listed in the Tables were purified to the 99+% level as judged by analytical HPLC, elemental analysis, and ^{1}H NMR (the remaining two were obtained >95% pure). This is illustrated in Figure 1 for 6'-O-methylsucrose (5) which was the end product of an 8-step synthetic sequence. In this case, 19 75-μl injections of a ~40% solution provided 537 mg of essentially 100% pure product.

ACKNOWLEDGMENT

The authors thank C. T. Seitz for technical assistance during the preliminary stages of this work.

REFERENCES

1. Crosby, G. A., DuBois, G. E., and Wingard, R. E., Jr., Drug Design, Vol. VIII, Ariëns, E. J., ed., Academic Press, New York, 1978, Chapter 5.

2. Crosby, G. A., DuBois, G. E., Hale, R. L. Stephenson, R. A., Wang, P. C., and Wingard, R. E., Jr., Sweeteners and Dental Caries, Roussos, G. G., and Shaw, J. H., eds., Information Retrieval, London, 1978, pp. 341-366.

3. Hough, L., and Phadnis, S. P., Nature (London), 263, 800 (1976).

4. Wingard, R. E., Jr., Ng, S., Dale, J. A., and Wang, P. C., unpublished results from these laboratories.

5. Swartz, M. L., and Furia, T. E., Food Technol. (Chicago), 31(11), 51 (1977).

6. DuBois, G. E., Crosby, G. A., Stephenson, R. A., and Wingard, R. E., Jr., J. Agric. Food Chem., 25, 763 (1977).

7. Whistler, R. L., and Wolfrom, M. L., eds., Methods in Carbohydrate Chemistry, Vol. I, Academic Press, New York, 1962, Section I.

8. Whistler, R. L. and BeMiller, J. N., eds., Methods in Carbohydrate Chemistry, Vol. VI, Academic Press, New York, 1972, Section I.

9. McGinnis, G. D., and Fang, P., J. Chromatogr., 130, 181 (1977).

10. Verhaar, L., and Dirkx, J., Carbohydr. Res., 59, 1 (1977).

11. Ball, D. H., Bissett, F. H., and Chalk, R. C., Carbohydr. Res., 55, 149 (1977).

12. Lindley, M. G., Birch, G. G., and Khan, R., Carbohydr. Res., 43, 360 (1975).

13. Hough, L., and Mufti, K. S., Carbohydr. Res., 25, 497 (1972).

14. Khan, R., Carbohydr. Res., 25, 504 (1972).

15. Belgian Patent 850180 (1977).

16. Fairclough, P. H., Hough, L., and Richardson, A. C., Carbohydr. Res., 40, 285 (1975).

17. Hough, L., Palmer, A. K., and Richardson, A. C., J. Chem. Soc., Perkin Trans. 1, 2513 (1972).