ELSEVIER

Journal of Chromatography A, 712 (1995) 149–154

JOURNAL OF CHROMATOGRAPHY A

Separation and detection of 4-hexadecylaniline maltooligosaccharide derivatives with packed capillary liquid chromatography–frit fast atom bombardment–mass spectrometry

Lena Johansson*, Hasse Karlsson, Karl-Anders Karlsson

Department of Medical Biochemistry, University of Göteborg, Medicinaregatan 9A, S-413 90 Göteborg, Sweden

Abstract

A LC–MS method is under development for the separation and detection of mixtures of native glycolipids and of oligosaccharide derivatives. The LC system is based on slurry-packed capillary columns. Frit fast atom bombardment (frit-FAB) is used as the LC–MS interface and ionisation technique and the column is connected to the frit via a 50 μm I.D. fused-silica capillary liner. Post column addition of matrix is achieved using a 50 μm I.D. fused-silica capillary liner with 2.5% (v/v) matrix solution. The two liners are joined through a septum and end side by side against the frit. The detection limit was found to be less than 1 pmole in the negative ion mode. A mixture of tetra to deca maltooligosaccharides reductively aminated with 4-hexadecylaniline (M_{4-10}-HDA) was separated on a straight phase silica column using gradient elution.

1. Introduction

The ionisation of native as well as synthetic glycolipids by fast atom bombardment (FAB) ionisation gives the advantage of intense molecular ions and characteristic fragmentation of the carbohydrate moiety [1]. Derivatisation followed by GC–MS of a mixture of glycolipids is a sensitive analytical tool but limited to glycolipids with up to four sugar units in the carbohydrate moiety [2]. Using a LC column connected to the frit-FAB ion source [3–6] a mixture of underivatised glycolipids can be separated and detected. Even though the sensitivity of the underivatised glycolipids in the negative ion mode is lower than for derivatised glycolipids in the positive ion mode [7], the amount of sample needed for detection is relatively low. Using packed capillary columns the amount of sample needed for separation, detection and analysis is further decreased since all off the injected sample is chromatographed and then delivered to the frit followed by FAB ionisation, Fig. 1. The use of a gradient mixing valve gives the possibility of gradient elution with optimal chromatographic performance.

The matrix is not included in the eluent because of its negative effects on the chromatographic behaviour; instead post-column addition of the matrix is used. At the column outlet a fused-silica capillary liner is connected using a zero dead-volume union. Another fused-silica

* Corresponding author.

0021-9673/95/$09.50
SSDI 0021-9673(95)00348-7

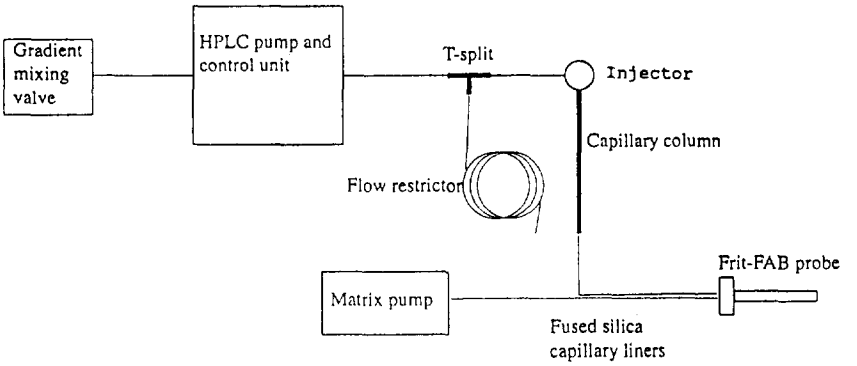

Fig. 1. Schematic of the LC and matrix systems.

capillary liner delivers the matrix solution and the two liners are joined at a septum and end side by side against the frit, Fig. 2. After solvent evaporation the sample is ionised by the matrix upon bombardment with xenon atoms. Mass spectra are continuously recorded and a mass chromatogram is displayed in real-time for either all or selected values of m/z.

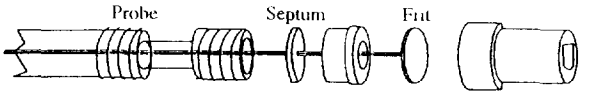

Fig. 2. Frit-FAB interface where one 50 μm fused-silica capillary liner connects the column with the frit and another 50 μm fused-silica capillary liner delivers the matrix solution. The two liners end side by side against the frit.

2. Experimental

2.1. Eluent and sample

The solvents used for the eluent [chloroform ($CHCl_3$, Fisons), methanol (CH_3OH, Fisons) and distilled water (H_2O)] were degassed using a continuous flow of helium through the solvent bottles.

The sample contained a mixture of tetra to deca maltooligosaccharides (M_{4-10}, Sigma) reductively aminated with 4-hexadecylaniline (HDA, Aldrich) [8,9] using dimetylsulfoxide (DMSO) as solvent during the reaction, Fig. 3. A 0.5-μl volume of the 4-hexadecylaniline maltooligosaccharide derivative (1.0 μg/μl M_{4-10}-HDA) was injected and separated on the col-

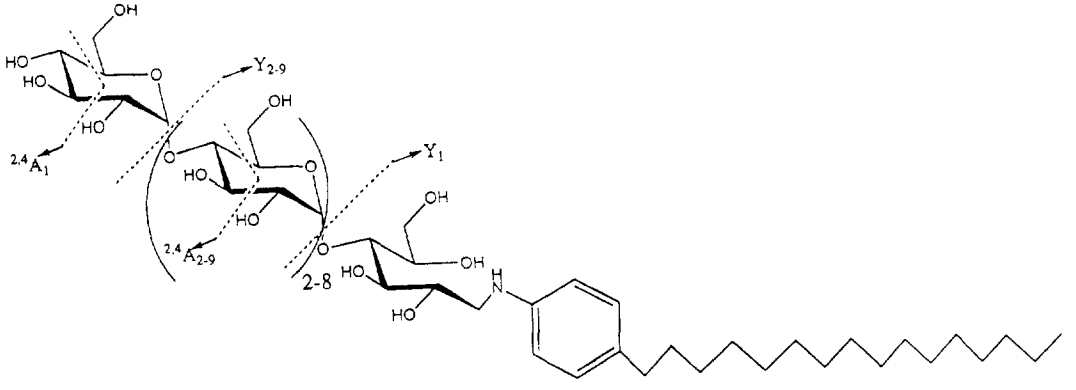

Fig. 3. Reductively aminated tetra to deca maltooligosaccharide (M_{4-10}-HDA) and the designations of ions that arise from fragmentations within the carbohydrate portion [1].

umn. Components M_{5-8}-HDA were present in less than 80 pmole/μl each while the M_4-HDA and the M_{9-10}-HDA were present in smaller amounts. The linear gradient used was $CHCl_3$–CH_3OH–H_2O 70:24:1 (v/v) to 60:38.5:1.5 (v/v) over a 10-min period.

Dilutions of N-acetyl-4-hexadecylaniline (ac-HDA) (280, 28, 2.8 and 1.4 pmole/μl) were prepared and chromatographed using isocratic elution with $CHCl_3$–CH_3OH–H_2O 70:24:1 (v/v).

2.2. Chromatographic system

The stainless steel capillary (25 cm × 250 μm I.D., Vici) was coupled up to a stainless steel packing reservoir (55 × 4.6 mm I.D.) for slurry packing [10,11]. The slurry was prepared from 15 mg of 5 μm straight phase silica particles (Kromasil 100 Å, EKA) in 1.2 ml acetonitrile–toluene–dioxane (2:1:1, v/v) and treated in an ultrasonic bath for a few minutes. The column was packed at 520 bar using an air-driven pump (Maximator LC 72) with heptane as compressor medium during 1.5 h.

A conventional HPLC pump (Pharmacia HPLC pump 2248) and control unit (Pharmacia LKP LCC 2252) delivered the eluent at 0.5 ml/min (Fig. 1). Before the injector the eluent flow was split by a T connection (Vici) where one way goes to the injector/column and the other was restricted by a 2 m × 100 μm I.D. fused-silica capillary, giving a total flow through the column of 3 μl/min. Gradient elution was achieved by the use of a gradient mixing valve connected to the pump control unit.

The injected volume was 0.5 μl by using a Rheodyne 7520 injector.

2.3. Matrix system

Negative matrix effects on the chromatographic behaviour were avoided by post-column addition of the matrix solution (Fig. 1). The matrix solution was delivered by a syringe pump (Harvard apparatus pump 22) at a flow-rate of 0.5 μl/min. When operating in the negative ion mode a 2.5% (v/v) solution of triethanolamine (TEA, Fluka) in CH_3OH–H_2O (1:1) was used.

2.4. The frit-FAB interface

A 50 μm I.D. fused-silica capillary liner was connected to the column outlet using a zero dead-volume union (Vici). Another liner delivers the matrix solution and the two liners are joined in a septum and end side by side at the frit (Fig. 2). The solvents evaporate at the frit leaving the sample dissolved in the remaining matrix where it is ionised by the bombardment.

2.5. Mass spectrometric conditions

Mass spectra were acquired on a JEOL SX-102A double-focusing mass spectrometer. In these experiments the mass spectrometer was operated in the negative ion mode, mass range scanned from 500 to 2000, total cycle time 14 s, FAB ionisation with xenon atoms at 6 keV and accelerating voltage at 10 kV. The resolution was set at 1000. During the detection of the smaller

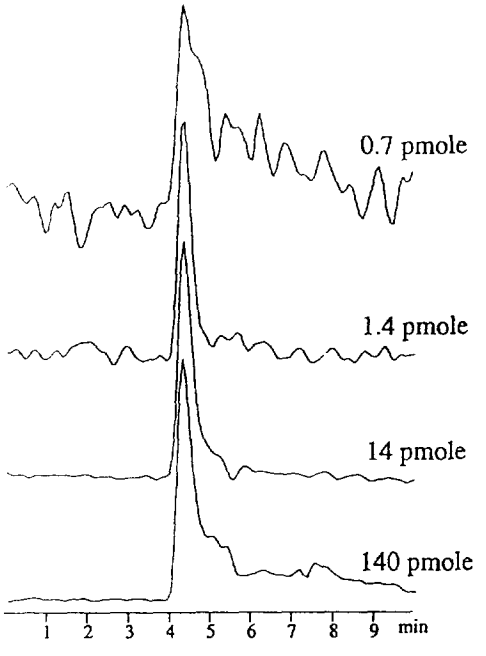

Fig. 4. Mass chromatograms of the pseudo-molecular ion at m/z 642 of ac-HDA for the different amounts analysed.

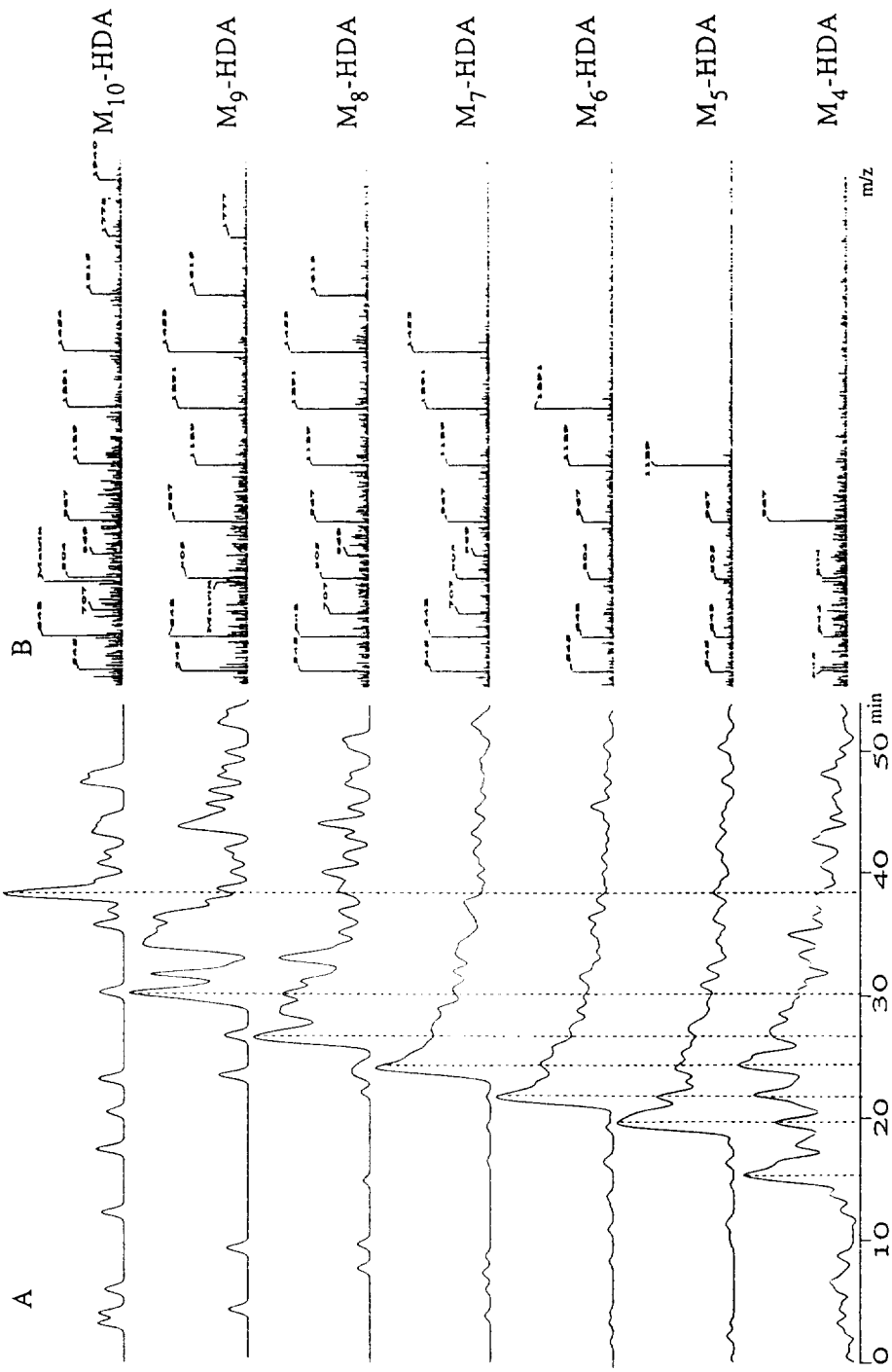

Fig. 5. (A) Mass chromatograms of the pseudo-molecular ion of each component in the mixture of M_{4-10}-HDA and (B) the corresponding mass spectrum for each component as an average over the major peak intensity with background subtraction by the scans between 1 and 4 min.

ac-HDA the mass range scanned was 100–400 with a total cycle time of 10 s. The ion source block was held at 48°C to prevent formation of ice-plugs in the frit and the liners during the evaporation of solvents. The ion source pressure was 10^{-4} Pa.

Mass chromatograms presented here (Figs. 4 and 5) are reconstructed ion chromatograms of the indicated m/z and the mass chromatograms for the M_{4-10}-HDA has been smoothed before presentation.

An average over the peak was chosen to represent the mass spectrum for the component. Background subtraction of the mass spectra was made using scans from the retention time between 1 and 4 min.

3. Results and discussion

3.1. Detection limits

In order to evaluate the detection limit of the system a dilution series of ac-HDA was used where 140, 14, 1.4 and 0.7 pmole respectively was injected (Fig. 4). The first three samples, 140, 14 and 1.4 pmole gave mass chromatograms and mass spectra with good signal-to-noise ratio. The 0.7 pmole sample resulted in a less well pronounced mass chromatogram and a weak mass spectrum. Therefore the detection limit was estimated to be 1 pmole in the negative ion mode with the system presented. The detection limit has also been investigated with M_2-HDA and gradient elution giving similar results. The given values are comparable with the results achieved in static FAB for underivatised glycolipids in the negative ion mode.

Ac-HDA was almost unretarded on the column with a retention time of 4 min which is approximately the value of t_0.

3.2. Separation and detection of a mixture of 4-hexadecylaniline maltooligosaccharide derivatives

The mass spectra of the 4-hexadecylaniline maltooligosaccharide derivatives (Fig. 5B and Table 1) are dominated by the Y fragment sequence ions (Table 2). Also present are some fragment ions due to cleavages within the pyranose rings, such as $^{2,4}A_4$, $^{2,4}A_5$ and $^{2,4}A_6$ [1] (Table 2).

Table 1
Pseudo-molecular ion masses, $[M-T]^-$, and retention times for the components in the analysed mixture

	$[M-H]^-$ (u)	Retention time (min)
M_2-HDA	967	15.6
M_5-HDA	1129	19.6
M_6-HDA	1291	22.6
M_7-HDA	1453	24.6
M_8-HDA	1615	26.9
M_9-HDA	1777	30.2
M_{10}-HDA	1939	33.5

The separation on the silica column is based on the length and polarity of the carbohydrate moiety. The chromatographic performance is difficult to evaluate from this sample since the sample is made from a homologous series of substituted maltooligosaccharides giving mass spectra with common Y fragment ions. The Y fragment ions of the higher homologues coincide with the pseudo-molecular ions of the lower homologues. This fact results in distorted mass chromatograms of pseudo-molecular ions (Fig. 5A). Still the retention time for each component

Table 2
Observed fragmentions of the components in the mixture

Fragment ion	m/z	Originated from
Y_3	642	all
Y_4	804	all
Y_5	967	M_{5-10}-HDA
Y_6	1129	M_{6-10}-HDA
Y_7	1291	M_{7-10}-HDA
Y_8	1453	M_{8-10}-HDA
Y_9	1615	M_{9-10}-HDA
Y_{10}	1777	M_{10}-HDA
$^{2,4}A_4$	545	all
$^{2,4}A_5$	707	M_{7-8}-HDA M_{10}-HDA
$^{2,4}A_6$	869	M_{7-8}-HDA M_{10}-HDA

The designations are indicated in Fig. 3.

is obtained and the differences are in the range of 2–4 min. Glycolipids derived from biological sources do not form a homologous series and the problem above does not occur. The aim of this work is to analyse native and synthetic glycolipids derived from different biological sources.

Acknowledgements

The sample of M_{4-10}-HDA was provided by Dr. Maria Ölwegård Halvarsson who is also acknowledged for valuable discussions and help. This work was supported by grants from The Swedish Technical Research Council.

References

[1] B. Domon and C.E. Costello, Glycoconjugate J., 5 (1988) 397.
[2] H. Karlsson, unpublished results.
[3] Y. Ito, T. Takeuchi, D. Ishii and M. Goto, J. Chromatogr., 346 (1985) 161.
[4] K. Sato, T. Kumazawa and Y. Katsumata, J. Chromatogr. A, 674 (1994) 127.
[5] M. Suzuki, M. Sekine, T. Yamakawa and A. Suzuki, J. Biochem., 105 (1989) 829.
[6] M. Suzuki, T. Yamakawa and A. Suzuki, J. Biochem., 108 (1990) 92.
[7] C.E. Costello and J.E. Vath, in J.A. McCloskey (Editor), Methods in Enzymology, Vol. 193, Mass Spectrometry, Academic Press, San Diego, CA, 1990, p. 738.
[8] M. Stoll, T. Mizuochi, R. Childs and T. Feizi, J. Biochem., 256 (1988) 661.
[9] H. Towbin, C.-A. Schoenenberger, D. Braun and G. Rosenfelder, Anal. Biochem., 173 (1988) 1.
[10] T. Tsuda and M. Novotny, Anal. Chem., 50 (1978) 271.
[11] Y. Hirata, M. Novotny, T. Tsuda and D. Ishii, Anal. Chem., 51 (1979) 475.