Application of High-Temperature High-Resolution Gas Chromatography to the Analysis of β-Cyclodextrin Derivatives

Alberto dos Santos Pereira, Edson Ferreira da Silva, and **Francisco Radler de Aquino Neto***

LADETEC, Chemistry Institute, Organic Chemistry Department, Rio de Janeiro Federal University, Centro de Tecnologia, Bloco A, Sala 607, Rio de Janeiro, RJ Brazil CEP 21949-900

Abstract

Short (3 m) columns are used with high-temperature stationary phases in analyses of reaction mixtures and isolated single species of β-cyclodextrin derivatives. The stationary phases are 5% phenyl–95% methylpolysiloxane and 30% diphenylpolysiloxane–40% sildiphenylene-ether–30% dimethyl polysiloxane; the latter phase is the best phase for the resolution of mixtures of positional isomers of β-cyclodextrin derivatives.

Introduction

β-cyclodextrin (β-CD) derivatives (Figure 1) are used as chiral stationary phases in high-resolution gas chromatography (HRGC) (1). Their use has resulted in the enantiomeric resolution of relevant molecules such as chiral precursors and synthetic intermediates of compounds with biological activity (2), simultaneous enantioselectivity of chiral flavors with different functionalities (3,4), determination of enantiomeric constituents in essential oils (5), resolution of chiral polychlorinated biphenyls (6), and other applications (7). Various CD derivatives described in the literature and used as chiral stationary phases are complex mixtures. They are synthesized by the stepwise replacement of hydroxyl groups, resulting in a considerable number of positional isomers. Because the quality of capillary chiral columns depends on the stationary phase purity (8), methods to analyze these complex mixtures are needed. This has been performed by nuclear magnetic resonance (NMR) (9), high-performance thin-layer chromatography (HPTLC) (10), and high-performance liquid chromatography (HPLC) (10–12).

The aims of this paper are to investigate the prospects of high-temperature HRGC (HTHRGC) for the analysis of underivatized β-CD derivatives with regard to, for example, fast analysis for reaction monitoring, elution temperature determination for potential chiral gas chromatography (GC) stationary phase applications, and use of Kováts indices and GC–mass spectrometry (GC–MS) fragmentography as a means of characterization. After the pioneering work of Koizumi et al. (10) using packed columns, Schomburg et al. used HTHRGC to analyze a few peralkylated CDs (11) and some alkyl derivatives of CDs after trimethylsilylation of the hydroxy groups (12). Koizumi et al. (10) also showed the potential of GC–MS to characterize substituted β-CDs (as partially methylated acetates).

Experimental

The various samples were synthesized in our laboratory according to methods described in the literature (13–18). They were 2,3,6-tri-*O*-methyl-β-CD, 2,3-di-*O*-methyl-6-*O*-dimethyl-

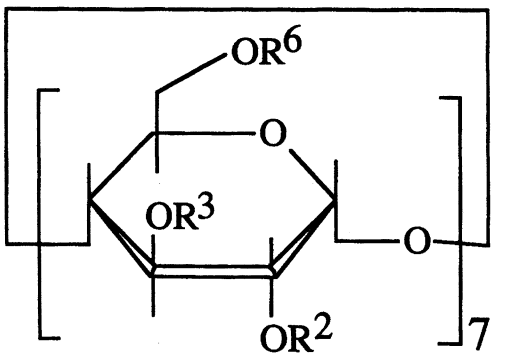

Figure 1. Structure of some β-CD derivatives. 2,3,6-tri-*O*-methyl-β-CD: $R^2 = R^3 = R^6 = CH_3$. 2,3-di-*O*-methyl-6-*O*-dimethyl-*tert*-butyl-silyl-β-CD: $R^2 = R^3 = CH_3$, R^6 = dimethyl-*tert*-butyl-silyl; 2,3-di-*O*-acetyl-6-*O*-dimethyl-*tert*-butyl-silyl-β-CD: $R^2 = R^3$ = acetyl; R^6 = dimethyl-*tert*-butyl-silyl.

* Author to whom correspondence should be addressed.

tert-butyl-silyl-β-CD, 2,3-di-*O*-acetyl-6-*O*-dimethyl-*tert*-butyl-silyl-β-CD, 2,6-di-*O*-methyl-3-*O*-acetyl-β-CD, and 2,3,6-tri-*O*-pentyl-β-CD. Along with these end products, the course of CD reactions was monitored by the analysis of untreated aliquots taken directly from the reaction mixture. Standards of 2,6-di-*O*-methyl-β-CD (mixture of positional isomers, Sigma, St. Louis, MO), tetracontane (*n*-C_{40}, Aldrich, Milwaukee, WI), and Polywax 655 (homologous series of high molecular weight; predominantly even carbon number; normal alkanes, 90% of which were between C_{30} and C_{80}) were obtained from Petrolite Specialty Polymers Group (Tulsa, OK).

HTHRGC

An on-column injector (Carlo Erba, Milano, Italy) was mounted on a Hewlett-Packard GC (model 5890 GC, Palo Alto, CA). High-temperature fused-silica capillary columns (3 m × 0.25-mm i.d.) were used; one was coated with DB5HT (5% phenylpolymethylsiloxane, 0.1-µm film, J&W Scientific, Rancho Cordova, CA), and the other was coated with Silaren-30 (30% diphenylpolysiloxane–40% sildiphenylene-ether–30% dimethylpolysiloxane, 0.1-µm film, BGB, Switzerland). Column performance was checked by the Grob test (19,20).

The temperature program began at 80°C (for 0.5 min), increased 20°C/min to 320°C, then increased 10°C/min to 400°C (for 10 min). The flame-ionization detector was operated at 400°C. Hydrogen was used as the carrier gas at a flow rate of 3.5 mL/min; the volume injected was 0.4 µL. Data were recorded with an HP model 3396-II (Palo Alto, CA) recording integrator.

HTHRGC–MS

The on-column injector (Carlo Erba) was mounted on a Hewlett-Packard model 5880 GC of a model 5897 GC–MS. A high-temperature fused-silica capillary column (3 m × 0.25-mm i.d.) coated with Silaren-30 (30% diphenylpolysiloxane–40% sildiphenylene-ether–30% dimethylpolysiloxane, 0.1-µm film) was used. The same temperature conditions used in the GC were used. The MS conditions were as follows: the interface temperature was 380°C, the source temperature was 300°C, the mode of ionization was electron impact (70 eV), and scanning was performed at 1 s/decade from 40 to 900 u.

HPTLC

Aluminum foil HPTLC (Alufolien, Kiesegel 60, Merck art. 7730, Darmstadt, Germany, 0.2-mm film thickness) was used with a chloroform–methanol–water (80:19:1) mobile phase. The sample was applied with a 1-µL Hamilton syringe mounted on a micrometer (spot diameter less than 0.5 mm) built in our laboratory. Visualization was realized by spraying a solution of ethanol and concentrated sulfuric acid (95:5) and heating to 100°C for 5 min.

Results and Discussion

HTHRGC

The HT columns that were used required additional conditioning for 1 h at 400°C to reduce column bleed to acceptable levels (21). Grob tests performed before and after conditioning

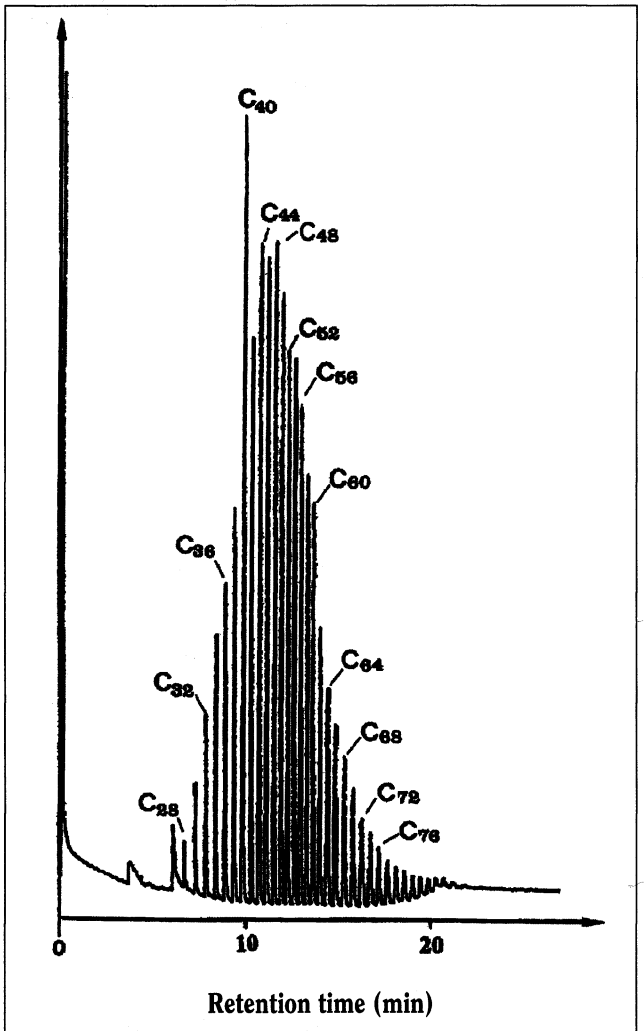

Figure 2. HTHRGC of Polywax 655 containing C_{40}.

Table I. Kováts Retention Indices of β-CD Derivatives

β-CD derivatives (MW)	Kováts index	
	Silaren-30	DB5HT
2,3,6-tri-*O*-methyl-β-CD (1428 u)	6213	5480
2,3-di-*O*-methyl-6-*O*-dimethyl-*tert*-butyl-silyl-β-CD (2128 u)	6210	6208
2,3-di-*O*-acetyl-6-*O*-dimethyl-*tert*-butyl-silyl-β-CD (2520 u)	peak 1: 7256 peak 2: 7437 peak 3: 7617	6800
2,6-di-*O*-methyl-β-CD (1442 u)	peak 1: 7010 peak 2: 7222 peak 3: 7421	undetermined (broad peak)*

* Kováts index "range": 5500–6400.

were similar, indicative of a reasonably inert capillary. The average separation number (TZ) between E10/E11 and E11/E12 was of the order 1.0/m.

The Kováts retention index was determined by coelution with Polywax 655 containing C_{40} (Figure 2), and the results are presented in Table I. It is interesting to note that despite the large molecular weights (M_r) of the CD derivatives (1400–2500 u), their retention characteristics were similar to C_{60}–C_{70} normal alkanes (M_r range, 844–984). This is probably due to their macrocyclic structure. The polar nature of the permethylated β-CDs can be assessed by their greater Kováts indices (Table I) in the more polar stationary phase (6213 in Silaren-30, as compared with 5480 in DB5HT). Substitution of the 6-*O*-methyl- for 6-*O*-dimethyl-*tert*-butylsilyl groups practically leveled off the indices (6210 in Silaren-30 and 6208 in DB5HT). This shows the importance of the six position (outside the CD cavity) to the intermolecular interactions. The use of short columns for analysis of β-CD derivatives in GC is very important because their elution temperatures in 3-m columns is about 330°C.

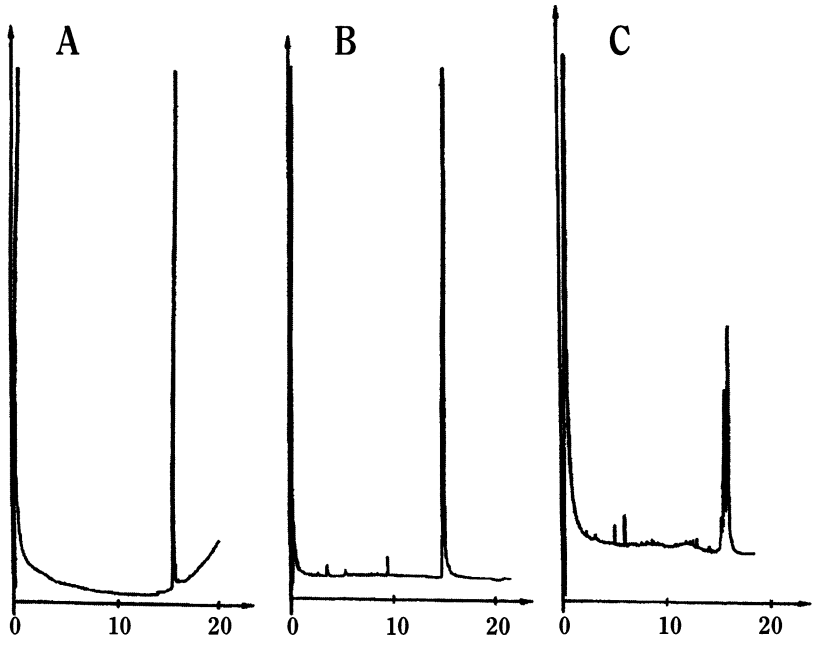

Figure 3. HTHRGC of β-CD derivatives in the Silaren 30 column: (A) 2,3,6-tri-*O*-methyl-β-CD, (B) 2,3-di-*O*-methyl-6-*O*-dimethyl-*tert*-butyl-silyl-β-CD, and (C) 2,3-di-*O*-acetyl-6-*O*-methyl-

Figure 3 shows the chromatograms of 2,3,6-tri-*O*-methyl-β-CD, 2,3-di-*O*-methyl-6-*O*-dimethyl-*tert*-butyl-silyl-β-CD, and 2,3-di-*O*-acetyl-6-*O*-dimethyl-*tert*-butyl-silyl-β-CD. The high resolution, together with symmetrical peaks obtained from the Silaren-30 column, makes HTHRGC an excellent tool for purity control in the syntheses of CD derivatives.

The analyses of β-CD derivatives by HTHRGC also gives as additional information the maximum temperature (T_{max}) of their use as stationary phases. If one were to use the cold trap concept (22), T_{max} would be approximately 100–120°C below the recorded elution temperature (Table II). Normally CD derivatives are used as chiral stationary phases dissolved in polysiloxanes. Taking DB5HT as an example, the T_{max} for 2,3,6-tri-*O*-methyl-β-CD would be around 200°C. This is in accordance with our experience and reported T_{max} for these columns (23–24).

Table II. Retention Times and Elution Temperatures of β-CD Derivatives

Derivative	Retention time (min)	Elution temperature (°C)
Silaren 30		
2,3,6-Tri-*O*-methyl-β-CD	15.120	351
2,3-Di-*O*-methyl-6-*O*-dimethyl-tert-butyl-silyl-β-CD	15.105	351
2,3-Di-*O*-acetyl-6-*O*-dimethyl-tert-butyl-silyl-β-CD	peak 1: 17.733 peak 2: 18.205 peak 3: 18.670	peak 1: 377 peak 2: 382 peak 3: 387
2,6-Di-*O*-methyl-β-CD	peak 1: 17.460 peak 2: 18.123 peak 3: 18.681	peak 1: 374 peak 2: 381 peak 3: 387
DB5HT		
2,3,6-Tri-*O*-methyl-β-CD	14.277	338
2,3-Di-*O*-methyl-6-*O*-dimethyl-*tert*-butyl-silyl-β-CD	15.683	352
2,3-Di-*O*-acetyl-6-*O*-dimethyl-*tert*-butyl-silyl-β-CD	17.146	367
2,6-Di-*O*-methyl-β-CD	14.3–16.1	338–356

NMR

β-CD derivatives have been characterized by proton and carbon NMR (^{1}H NMR and ^{13}C NMR). The 2,3,6-tri-*O*-methyl-β-CD was characterized through ^{1}H NMR by the presence of three singlets that correspond to three methoxy groups in C-2 (MeO-2, 3.68 ppm, 21H), C-3 (MeO-3, 3.54 ppm, 21H), and C-6 (MeO-6, 3.42 ppm, 21H) and through ^{13}C NMR by the presence of signals in 61.1, 58.2, and 58.6 ppm (C-2, C-3, and C-6, respectively). 2,3-di-*O*-methyl-6-*O*-dimethyl-*tert*-butyl-silyl-β-CD has four relevant singlets, C-2 (MeO-2, 3.68 ppm, 21H), C-3 (MeO-3, 3.52 ppm, 21H), 0.88 ppm (63H, -C[CH_3]$_3$), and 0.03 ppm (42H, [CH_3]$_2$Si).

This demonstrates that the 2,3,6-tri-*O*-methyl-β-CD and 2,3-di-*O*-methyl-6-*O*-dimethyl-*tert*-butyl-silyl-β-CD synthesized in our laboratory are compatible with literature data (25,26). The absence of byproducts was confirmed with HTHRGC (e.g., Figure 3). The reaction mixture of the syntheses of 2,3-di-*O*-

acetyl-6-*O*-dimethyl-*tert*-butyl-silyl-β-CD was also analyzed, and it showed at least three different products (Figure 3C). In ^{1}H NMR, two singlets (2.06 and 2.07 ppm), characteristic of the acetyl group hydrogens, were expected together with one singlet (0.88 ppm) characteristic of the *tert*-butyl group hydrogens (26). In our reaction mixture, other signals (0.88- and 2.07-ppm regions) confirmed the presence of byproducts that could not be identified (Figure 4).

HTHRGC versus HPTLC and NMR

The analysis of 2,6-di-*O*-methyl-3-*O*-acetyl-β-CD by HTHRGC (Figure 5) was more informative than that by HPTLC. As an example, this technique showed only one spot in the follow-up of the conversion of 2,6-di-*O*-methyl-β-CD to 2,6-di-*O*-methyl-3-*O*-acetyl-β-CD (after 12 h, one spot corresponding to a retardation factor [R_f] of 0.74; after 36 h, a spot at R_f 0.62; and after 60 h, a spot at R_f 0.62). On the other hand, HTHRGC distinctly showed a broad peak after 12 h of reaction, two large peaks after 36 h, and quite a different scenario after 60 h (Figure 5).

NMR is excellent for the characterization of intermediates and final products (e.g., the 60-h sample was also characterized as a mixture by the presence of a multiplet from the acetyl groups [δ, 2.06–2.07 ppm]). However, it would not be useful to follow the progress of the reaction because a considerable amount would have to be transferred, contrary to the minute quantities needed for HRGC. Also, HRGC is very fast, especially using cold on-column injection, in which the raw product can be analyzed without derivatization.

Polarity and selectivity

Silaren 30, a medium-polarity phase, separated three peaks of 2,6-di-*O*-methyl-β-CD, whereas DB5HT (low polarity), gave one large peak. This demonstrates the influence of polarity in the resolution of positional isomers of β-CDs (Tables I and II, Figure 6).

The result of the Silaren-30 analysis of 2,6-di-*O*-methyl-β-CD is very important for substantiating the applicability of HTHRGC to CD analyses. This data confirms the results of Koizumi et al. (10) and Spencer et al. (9), who showed by TLC, HPLC, and NMR spectroscopy, that the synthetic procedures of Boger et al. (27) and Szejtli et al. (28) give a mixture of 2,6-di-*O*-methyl-β-CD and hexakis(2,6-di-*O*-methyl)mono(2,3,6-tri-*O*-methyl)-β-CD.

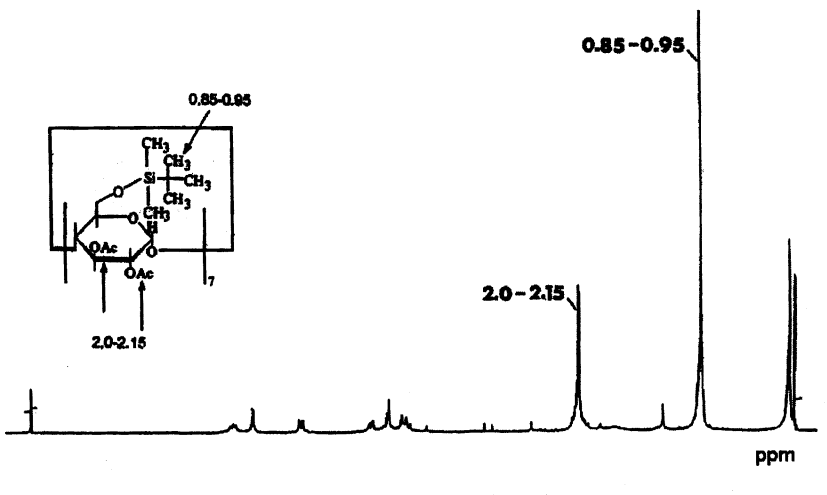

Figure 4. ^{1}H NMR spectrum of 2,3-di-*O*-acetyl-6-*O*-methyl-β-CD.

GC–MS

HTHRGC–MS was used for identification of the β-CD derivatives. They presented a fragmentation pattern characteristic of oligosaccharides (29). This can be used, for example, for the characterization of 2,3,6-tri-*O*-methyl-β-CD and 2,3-di-*O*-methyl-6-*O*-dimethyl-*tert*-butyl-silyl-β-CD, as shown in Figure 7. The β-CD derivatives produced low-mass fragment ions due to cleavage of the pyranosidic rings. 2,3,6-Tri-*O*-methyl-β-CD gave a diagnostic fragment ion (*m/z* 188), and 2,3-di-*O*-methyl-6-O-dimethyl-*tert*-butyl-silyl-β-CD produced a fragment ion (*m/z* 288). Further mass spectral data from pure samples by probe MS is available elsewhere (29) and could be used as a reference for the interpretation of HTHRGC–MS work on CD mixtures.

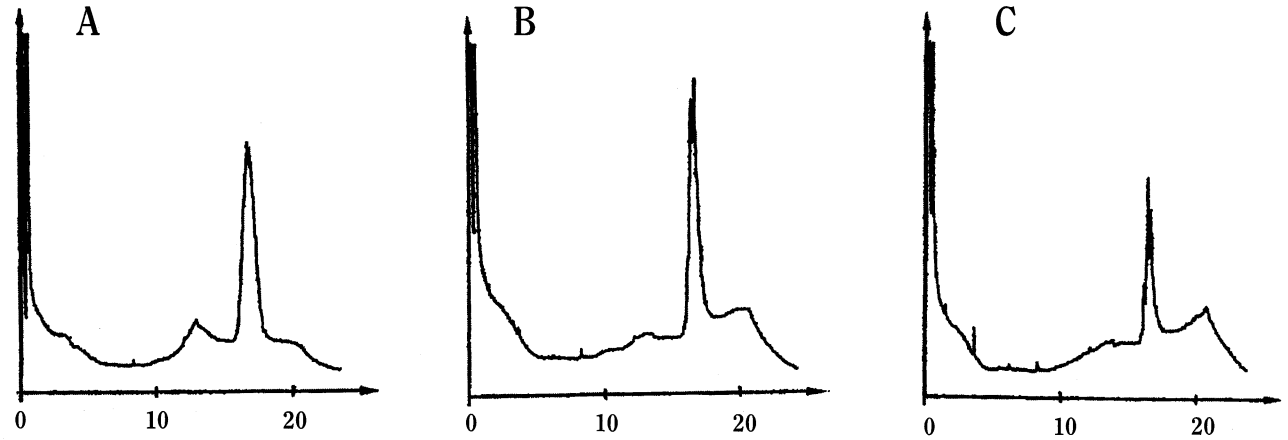

Figure 5. HTHRGC of the follow-up of the syntheses for 2,6-di-*O*-methyl-3-*O*-acetyl-β-CD (A) after 12 h of reaction, (B) after 36 h of reaction, and (C) after 60 h of reaction.

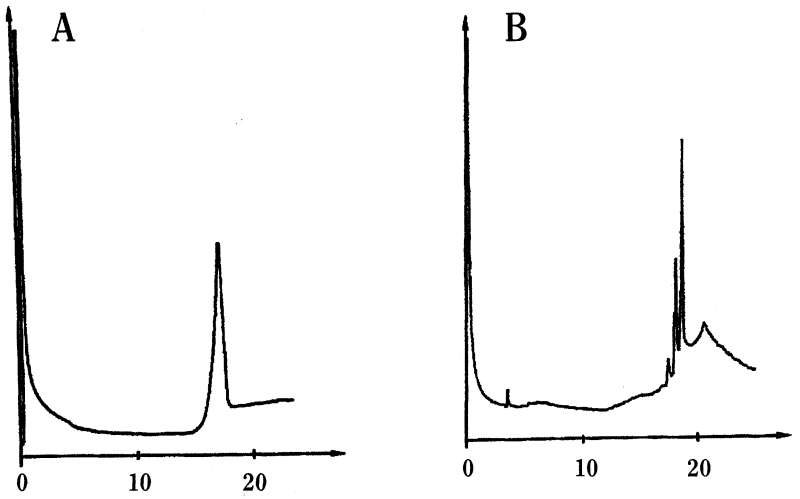

Figure 6. HTHRGC of 2,6-di-*O*-methyl-β-CD: (A) Silaren 30 and (B) DB5HT.

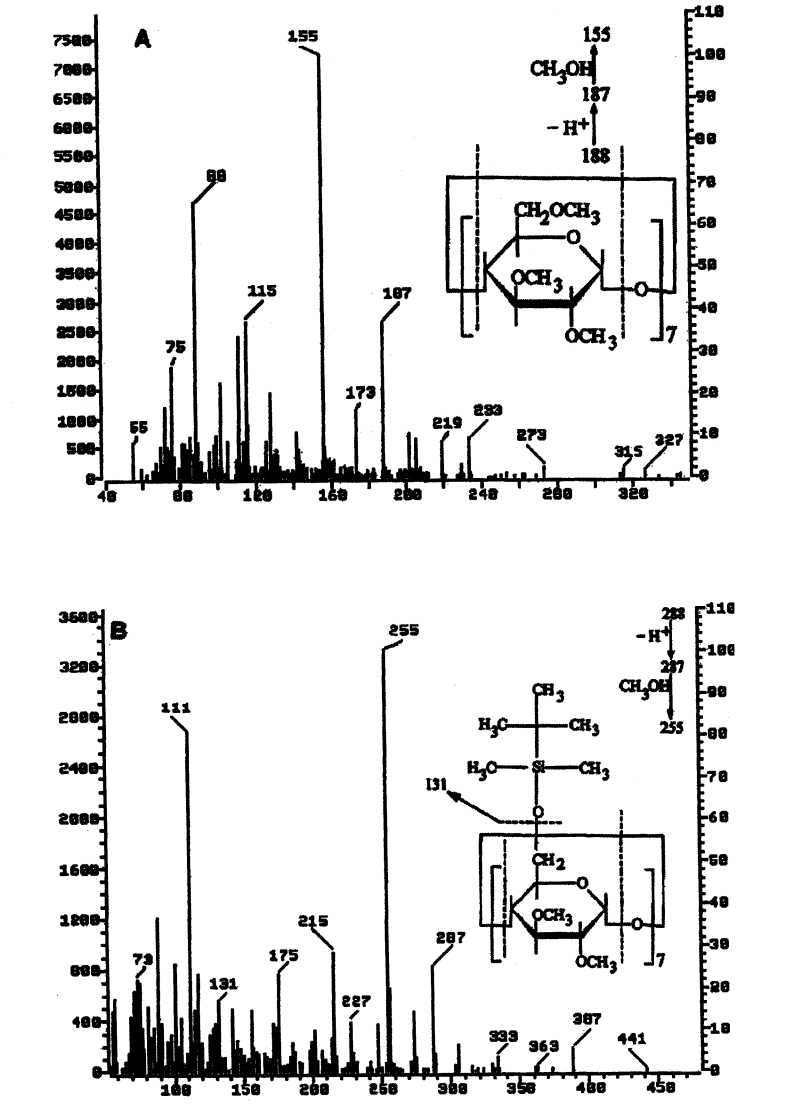

Figure 7. Typical electron impact mass spectra from HTHRGC–MS: (A) 2,3,6-tri-*O*-methyl-β-CD and (B) 2,3-di-*O*-methyl-6-*O*-dimethyl-*tert*-butyl-silyl-β-CD.

Conclusion

HTHRGC can be used for the analyses of isolated single species and reaction mixtures of β-CD derivatives; it has shown to be an excellent routine method for following their synthesis. It presents some distinguished features such as a lower cost and higher resolution than HPLC, faster response and lower sample amount than NMR, and more information than HPTLC. The technique allows for the anticipation of T_{max} for capillary columns to be prepared with CD derivatives. HTHRGC–MS can also be used for characterization of CD derivatives. When resolution is not the major concern, use of thin-film, short capillary columns (< 5 m) brings elution temperatures almost within the range of HRGC.

Acknowledgments

The authors wish to thank SR-2/UFRJ, CNPq, FUJB, and Finep for financial support.

References

1. E.F. Silva, M.C.K.W. Ramos, and F.R. Aquino Neto. Utilizacao de derivados das ciclodextrinas como fase estacionaria quiral em cromatografia gasosa de alta resolucao [Cyclodextrin derivatives as chiral stationary phases in HRGC]. *Química Nova* **19:** 473–79 (1996).
2. C.P. Granville, B. Gehreke, W.A. König, and J.W. Wainer. Determination of the enantiomers of isofosfamide and its Z- and E-*N*-dichloroethylated metabolites in plasma and urine using enantioselective gas chromatography with mass spectrometric detection. *J. Chromatogr.* **622:** 21–31 (1993).
3. A. Dietrich, B. Mass, and V. Karl. Stereoisomeric flavor compounds, Part IV: stereodifferentiation of some chiral volatiles on heptakis(2,3-di-*O*-acetyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin. *J. High Res. Chromatogr.* **15:** 176–79 (1992).
4. A. Mosandl, K. Rettinger, K. Fischer, V. Schubert, H.G. Schmarr, and B. Maas. Stereoisomeric flavor compounds. XLI. New applications of permethylated beta-cyclodextrin phase in chiral CGC analysis. *J. High Res. Chromatogr.* **13:** 382–90 (1990).
5. W.A. König, A. Rieck, I. Hardt, B. Gehrcke, K. Kubecka, and K. Muhle. Enantiomeric composition of the chiral constituents of essential oils, Part 2: Sesquiterpene hydrocarbons. *J. High Res. Chromatogr.* **17:** 315–20 (1994).

6. W.A. König, B. Gehrcke, T. Runge, and C. Wolf. Gas chromatographic separation of atropisomeric alkylated and polychlorinated biphenyls using modified cyclodextrins. *J. High Res. Chromatogr.* **16:** 376–78 (1993).
7. H. Casabianca, J.B. Graff, P. Jane, and C. Perrucchiett. Application of hyphenated techniques to the chromatographic authentication of flavors in food products and perfumes. *J. High Res. Chromatogr.* **18:** 279–85 (1995).
8. A.S. Pereira, E.F. Silva, and F.R. Aquino Neto. Cromatografia gasosa de alta temperatura: a nova fronteira e sua aplicacao na analise de compostos de alta massa molecular [High temperature gas chromatography: the new frontier and its application to the analysis of high molecular weight compounds]. *Química Nova* **19:** 600–604 (1996).
9. C.M. Spencer, J.F. Stoddart, and R. Zarzycki. Structural mapping of an unsymmetrical chemically modified cyclodextrin by high-field nuclear magnetic resonance spectroscopy. *J. Chem. Soc. Perkin Trans.* **2:** 1323–36(1987).
10. K. Koizumi, Y. Kubota, T. Utamura, and S. Horiyama. Analysis of heptakis(2,6-di-*O*-methyl)-β-cyclodextrin by thin-layer, high performance liquid and gas chromatography. *J. Chromatogr.* **368:** 329–37 (1986).
11. G. Schomburg, A. Deege, H. Hinrichs, E. Hubinger, and H. Husmann. Preparation, purification, and analysis of alkylated cyclodextrins. *J. High Res. Chromatogr.* **15:** 579–84 (1992).
12. A. Deege, H. Husmann, E. Hübinger, F. Kobor, and G. Schomburg. Purification and analysis of partially alkylated cyclodextrins by liquid and gas chromatography. *J. High Res. Chromatogr.* **16:** 587–93 (1993).
13. V. Schurig, M. Jung, D. Schmalzing, M. Scheimer, J. Duvekot, J.C. Buyten, J.E. Peene, and P. Mussche. CGC enantiomer separation on diluted cyclodextrin derivatives coated on fused silica columns. *J. High Res. Chromatogr.* **13:** 470–474 (1990).
14. P. Fugedi. Synthesis of heptakis(6-*O*-*tert*-butyldimethylsilyl) cyclomaltoheptaose and octakis(6-*O*-*tert*-butyldimethylsilyl) cyclomaltooctaose. *Carbohydr. Res.* **192:** 366–69 (1989).
15. W.A. König, D. Icheln, T. Runge, I. Pforr, and A. Krebs. Cyclodextrins as chiral stationary phases in capillary gas chromatography. *J. High Res. Chromatogr.* **13:** 702–707 (1990).
16. K. Takeo, K. Uemura, and H. Mitoh. Derivatives of α-cyclodextrin and the synthesis of 6-*O*-β-D-glucopyranosyl-β-cyclodextrin. *J. Carbohydrate Chem.* **7:** 293–308 (1988).
17. K. Kein, A. Kohnes, R. W. Meltzon, and H. Romer. Enantiomer separation by gas chromatography on cyclodextrin chiral stationary phases. *J. High Res. Chromatogr.* **14:** 507–29 (1991).
18. G. Wenz, P. Mischnick, R. Krebbe, and M. Richters. Preparation and characterization of per-*O*-pentylated cyclodextrins. *J. High Res. Chromatogr.* **13:** 724–28 (1990).
19. K. Grob Jr., G. Grob, and K. Grob. Testing capillary gas chromatographic columns. *J. Chromatogr.* **156:** 1–20 (1978).
20. K. Grob, G. Grob, and K. Grob Jr. Comprehensive standardized quality test for glass capillary columns. *J. Chromatogr.* **219:** 13–20 (1981).
21. F.R. Aquino Neto, J.N. Cardoso, A.S. Pereira, M.C.Z. Fernandes, C.A. Caetano, and A.L.C. Machado. Application of high temperature high resolution gas chromatography to paraffinic deposits in petroleum production pipelines. *J. High Res. Chromatogr.* **17:** 259–63 (1994).
22. K. Grob. *Classical Split and Splitless Injection in Capillary Gas Chromatography; with Some Remarks on PTV Injection.* Huethig, Heidelberg, Germany, 1993, p. 547.
23. V. Schurig and H.P. Nowotny. Separation of enantiomers on diluted permethylated β-cyclodextrin by high resolution gas chromatography. *J. Chromatogr.* **441:** 155–63 (1988).
24. H.P. Nowotny, D. Schmalzing, D. Wistuba, and V. Schurig. Extending the scope of enantiomer separation on diluted methylated β-cyclodextrin derivatives by high-resolution gas chromatography. *J. High Res. Chromatogr.* **12:** 383–93 (1989).
25. V. Schurig, M. Jung, D. Schmalzing, M. Schleiner, J. Duvekot, J.C. Buyten, J.A. Peene, and P. Mussche. CGC enantiomer separation on diluted cyclodextrin derivatives coated on fused silica columns. *J. High Res. Chromatogr.* **13:** 470–74 (1990).
26. K. Takeo, H. Mitoh, and K. Ueumura. Selective chemical modification of cyclomaltooligosaccharides via *tert*-butyldimethylsilylation. *Carbohydr. Res.* **187:** 203–21 (1989).
27. J. Boger, R.J. Corcoran, and J.M. Lehn. Cyclodextrin chemistry selective modification of all primary hydroxyl groups of alfa- and beta-cyclodextrins. *Helv. Chim. Acta* **61**: 2190–2218 (1978).
28. J. Szejtli, A. Lipták, I. Jodál, P. Fügedi, P. Nánási, and A. Neszmélyi. Synthesis and ^{13}C-NMR spectroscopy of methylated beta-cyclodextrins. *Staerke* **32:** 165–69 (1980).
29. N.G. Carlsson, H. Karlsson, and A.S. Sandberg. Determination of oligosaccharides in foods, diets, and intestinal contents by high-temperature gas chromatography and gas chromatography mass spectrometry. *J. Agric. Food Chem.* **40:** 2404–12 (1992).

Manuscript accepted November 17, 1997.