Journal of Chromatography, 625 (1992) 157–161
Elsevier Science Publishers B.V., Amsterdam

CHROM. 24 536

Determination of the impurity profile of γ-cyclodextrin by high-performance liquid chromatography

Gregory White*, Thomas Katona, Julius P. Zodda and Michael N. Eakins

Bristol-Myers Squibb Pharmaceutical Research Institute, One Squibb Drive, P.O. Box 191, New Brunswick, NJ 08903-0191 (USA)

(First received February 6th, 1992; revised manuscript received June 15th, 1992)

ABSTRACT

High-performance liquid chromatography (HPLC) was used to determine the impurity profile of γ-cyclodextrin (γ-CD), a compound used as a stabilizer in pharmaceutical formulations. An HPLC assay was developed which separated γ-CD from potential impurities which included 7 linear (glucose to maltoheptaose) and 2 unbranched cyclic (α- and β-CD) glucose polymers. The method employed a mobile phase consisting of acetonitrile–water (73:27), a Whatman 5-μm Partisil PAC column (250 × 4.6 mm I.D.), refractive index detection, and achieved a resolution (R_s) ≥ 1.5 for all impurities in the presence of γ-CD. The method presented provided improvements over existing methods by achieving a rapid separation (< 25 min) of compounds not previously reported using moderate temperature control and quantitated impurities at the 0.1–1.2% (w/w) level of sensitivity.

INTRODUCTION

γ-Cyclodextrin (γ-CD), a cyclic octamer of D-glucose with α-(1,4)-linkages, is a water soluble compound which can form reversible complexes with poorly water soluble molecules resulting in soluble molecular inclusion complexes. This "host–guest" relationship can substantially increase the aqueous solubility of many pharmaceuticals [1–3], and act as a stabilizer by reducing rates of oxidation, decomposition, disproportionation, polymerization, and autocatalytic reactions [4]. CardioTec, a kit for the preparation of the radiopharmaceutical myocardial imaging agent Tc^{99m} teboroxime, contains γ-CD to improve the stability of the formulation. It was necessary to establish the impurity profile of γ-CD to monitor both the quality and the manufacturer to manufacturer equivalence of the material.

Commercially, γ-CD is produced from the enzymatic digestion of starch. The γ-CD is selectively purified from the crude digest containing linear glucose polymers, α- (cyclic hexamer), β- (cyclic heptamer), γ- and higher cyclodextrins [5]. An analytical method was needed to determine the level of potential linear and unbranched cyclic polymeric glucose impurities in γ-CD. Chemical structures for γ-CD and some potential impurities are shown in Fig. 1.

Various high-performance liquid chromatographic (HPLC) methods have been applied for carbohydrate analysis using such columns as μBondapak-carbohydrate [6–9], aminopropyl-bonded [10–12], aminocyano-bonded [13], specialized C_8 and C_{18} [14–18], silica [19,33], and ion exchange columns [20–32], with some investigators using a mobile phase amine modifier to affect separation [8,19,33]. Several means of detection have been employed including refractive index [6–16,19,24,30,34], pulsed amperometric [17,32], and indirect UV–vis detection with micro HPLC [18]. Koizumi *et al.* [34] compared four different amino bonded columns to successfully separate some, but not all, of the components addressed in this study, and needed highly elevated temperatures (75°C) to eliminate anomeric peak

Correspondence to: Gregory White, Bristol-Myers Squibb Pharmaceutical Research Institute, One Squibb Drive, Building 80, Room 43, P.O. Box 191, New Brunswick, NJ 08903-0191, USA.

0021-9673/92/$05.00

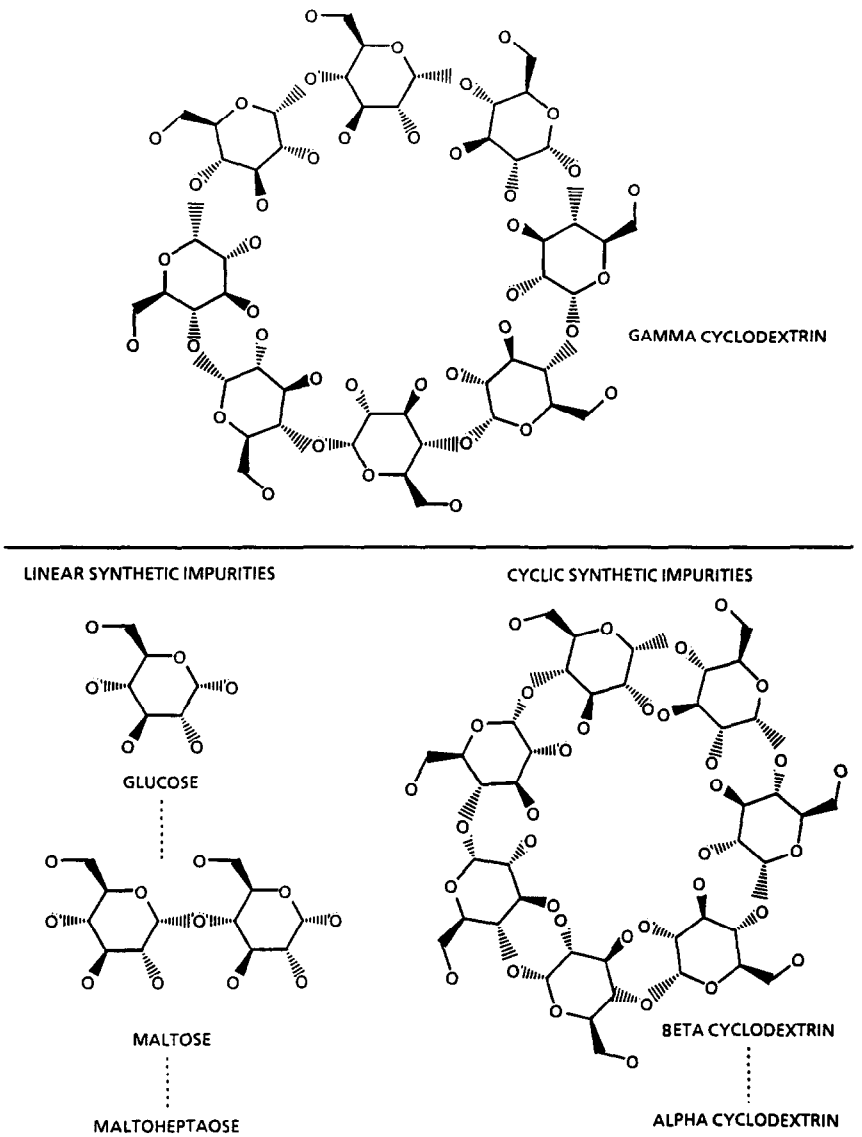

Fig. 1. Structures for γ-CD and some potential synthetic pathway impurities.

splitting on one of the columns. Rabel *et al.* [13] contended that the mode of separation for carbohydrates using polar bonded phases such as Partisil PAC is normal-phase partition chromatography since retention times are decreased by increasing the water content of the mobile phase. Historically, the most useful polar bonded phases for normal-phase separations have been ether, cyano, and amino [13]. Partisil PAC, consisting of alkyl groups containing amino-cyano groups, provides a combination of these features and was chosen as the optimal material for our analysis.

This paper details the separation of 7 linear (glucose to maltoheptaose) and 3 unbranched cyclic (α-, β- and γ-CD) glucose polymers in less than 25 min at 45°C and reports limits of detection, response factors and quantitative impurity results obtained utilizing refractive index detection.

EXPERIMENTAL

Materials

γ-CD was obtained from Advanced Separation Technologies, Astec (Whippany, NJ, USA), American Maize-Products Company, Amaizo (Hammond, IN, USA) and Fluka Chemika-BioChemika (Buchs, Switzerland). α- and β-CD and linear glucose polymers were obtained from Fluka, and Sigma Chemical Company (St. Louis, MO, USA). The acetonitrile was HPLC grade from Baxter Scientific Products (McGaw Park, IL, USA). The water was HPLC/organic free from the NANOpure II system from SYBRON/Barnstead (Boston, MA, USA).

The HPLC/data collection system consisted of a Spectra-Physics Model 4270 integrator, Model 8800 pump, and Model 8780 autosampler with a 10-μl (nominal) fixed loop injector all on Spectra-Physics LABNET from Spectra-Physics (Piscataway, NJ, USA), a Fiatron Model CH-30 column heater from Rainin Instrument (Woburn, MA, USA), and a Hewlett-Packard Model 1047A refractive index detector from Hewlett-Packard (Piscataway, NJ, USA) maintained at 45°C. A Whatman Partisil 5 PAC, 5 μm, 250 mm × 4.6 mm I.D. column from Baxter maintained at 45°C was used with a mobile phase consisting of acetonitrile–water (73:27) pumped at a flow-rate of 1.0 ml/min.

Methods

Prior to use, the column was conditioned by pumping mobile phase at 1.0 ml/min for 24 h. A solution containing 1 mg/ml each of γ-CD and maltohexaose in warm (50°C) acetonitrile–water (60:40) was injected for system suitability. The resolution, $R_s = 2(t_2 - t_1)/(W_1 + W_2)$, between the peaks should be ≥ 1.5.

For impurity determination, stock sample solutions (100 mg/ml) were prepared in warm (50°C) water. Working sample solutions (40 mg/ml) were prepared in warm (50°C) acetonitrile–water (60:40) and injected as soon as possible to prevent precipitation.

Samples were injected in duplicate and the % impurity index was calculated on an area % basis.

RESULTS AND DISCUSSION

A chromatogram of an injection of γ-CD and nine possible impurities (5 mg/ml) produced baseline resolution of all the components, $R_s \geq 1.5$ (Fig. 2). The resolution between γ-CD and maltohexaose, the closest eluting component, was 2.2. This separation represents a notable improvement over a comparable separation reported by Koizumi *et al.* [34], wherein resolution between γ-CD, maltopentaose, maltohexaose and α- and β-CD was inadequate or unreported. Relative response factors, limits of detection ($S/N = 2$), and retention times are given in Table I. Relative response factors were equivalent and limits of detection were ≤0.3 (% w/w) for the α-, β- and γ-CD. However, the response factors for the linear glucose polymers increased with increasing retention time and limits of detection were the most sensitive for the fast eluting, sharp peaks (*i.e.*, glucose, maltose) and were least sensitive for the

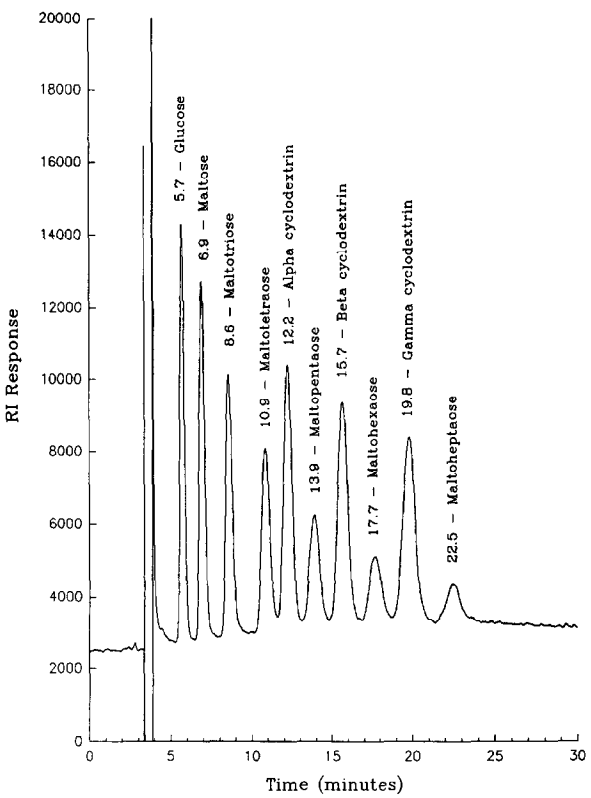

Fig. 2. Chromatogram of γ-CD and nine potential synthetic pathway impurities (10.0 μl injection containing 5 mg/ml each) using a Partisil PAC column (45°C), acetonitrile–water (73:27) at 1.0 ml/min, and refractive index detection (45°C).

TABLE I

HPLC REFRACTIVE INDEX DETECTION PARAMETERS FOR γ-CD AND IMPURITIES

Compound	Relative response factor[a]	Detectable weight on column (μg)[b]	Limit of detection (% w/w)[c]	Retention time (min)
Glucose	1.30	0.3	0.1	5.7
Maltose	1.35	0.6	0.2	6.9
Maltotriose	1.39	1.2	0.3	8.6
Maltotetraose	1.62	1.3	0.3	10.9
α-CD	1.00	0.6	0.2	12.2
Maltopentaose	1.87	2.5	0.6	13.9
β-CD	1.03	1.3	0.3	15.7
Maltohexaose	2.28	4.9	1.2	17.7
γ-CD	1.00	1.3	0.3	19.8
Maltoheptaose	2.97	4.8	1.2	22.5

[a] Relative to γ-CD as (compound concentration/compound area)/(γ-CD concentration/γ-CD area).
[b] When signal-to-noise ratio equals 2 ($S/N = 2$).
[c] Relative to maximum injectable amount of 400 μg γ-CD on the column.

long eluting, broad peaks (*i.e.*, maltohexaose, maltoheptaose). Although refractive index detection lacks the sensitivity of techniques such as pulsed amperometric detection [32], the level of sensitivity was sufficient (0.1–1.2% w/w) to provide a satisfactory impurity profile of the nine potential components of interest.

TABLE II

POTENCY AND IMPURITY INDEX RESULTS FROM TWO MANUFACTURERS OF γ-CD

Sample number	Manufacturer	Potency (% anhy.)	Impurity index (%)	Impurities found
1	I	100.0	0.00	None
2	I	99.4	0.00	None
3	II	101.0	0.00	None
4	II	–	0.65	α-, β-CD
5	II	97.8	0.00	None
6	II	98.6	0.52	α-, β-CD
7	II	99.6	0.00	None
8	II	102.0	0.00	None
9	I	100.1	0.00	None
10	II	101.1	0.00	None

To show applicability of the method, multiple samples of γ-CD raw material from two manufacturers (I and II) were assayed for total impurity index (Table II). Of 10 lots assayed, none showed any of the linear glucose impurities; however, two lots contained small amounts (< 1% w/w) of α- and β-CD impurities. The practical application of the method is graphically displayed in Fig. 3, showing overlaid chromatograms of γ-CD raw materials containing (A) α- and β-CD impurities at a level (0.65% impurity index) near the detection limit and (B) no impurities. In addition, the method was utilized to determine the potency of the 10 lots of γ-CD (Table II).

The sensitivity and accuracy of the method was demonstrated using α-CD (relative response factor = 1) as a model impurity. A 102% recovery of

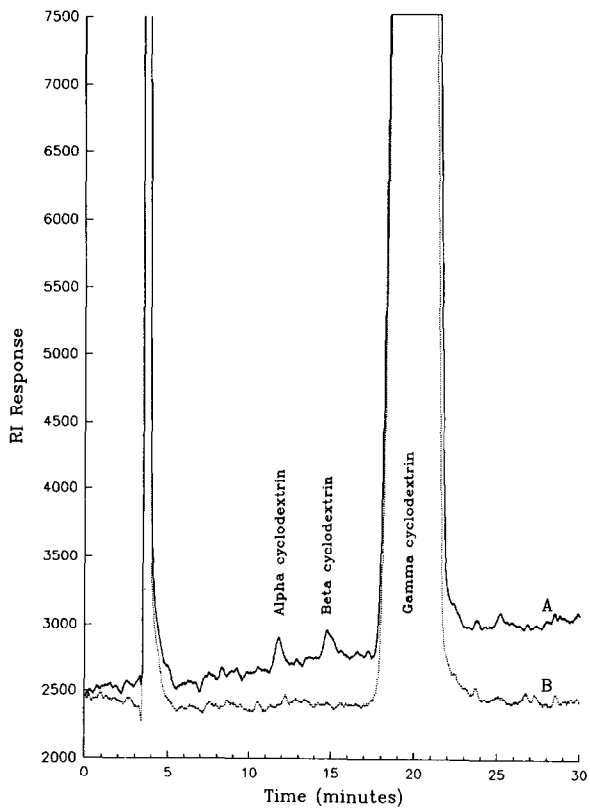

Fig. 3. Chromatograms of γ-CD raw materials containing (A) α- and β-CD impurities (at 0.65% impurity index) and (B) no impurities, using Partisil PAC column (45°C), acetonitrile–water (73:27) at 1.0 ml/min, and refractive index detection (45°C).

α-CD was obtained when γ-CD was spiked with 1% (w/w) α-CD.

The highest concentration of γ-CD in acetonitrile–water (60:40) injected was 40 mg/ml. Higher concentrations produced solubility problems. Acetonitrile–water (60:40) was chosen as an intermediate solvent between water and the mobile phase [acetonitrile–water (73:27)]. The injection solution, column, and detector were maintained at 45–50°C to enhance and stabilize the chromatography as well as to maintain the solubility of γ-CD. The optimized chromatographic conditions utilizing moderate temperature control and a mobile phase of high organic/aqueous ratio (≈3:1) produced Gaussian shaped peaks and eliminated anomeric peak splitting, a concern with other methods [34].

Ruggedness testing indicated that after approximately 250 injections were made on the column over a period of 16 days, column performance was reduced. The use of a short water rinse time (10 min) for column clean-up is recommended to eliminate possible column voids or channeling due to dissolution of silica. The use of a silica saturator column may also be employed.

In conclusion, this HPLC method provides a profile of 7 linear (glucose through maltoheptaose) and 2 unbranched cyclic (α- and β-CD) glucose polymer impurities in the presence of γ-CD. The method is rapid (<25 min) and sensitive for quantitating impurities at the 0.1–1.2% (w/w) level, and is also suitable for use as a raw material potency assay due to its ability to eliminate potential impurity interferences. In addition, the method potentially can be used to monitor the quality of γ-CD used as a mobile phase additive for chiral separations.

ACKNOWLEDGEMENTS

The author thanks Dr. Joel Kirschbaum for his helpful comments, Karen Skupeika for her help with the structural figure and is grateful to the Bristol-Myers Squibb Library and Literature services for their assistance.

REFERENCES

1 M. E. Brewster, J. W. Simpkins, M. S. Hora, W. C. Stern and N. Bodor, *J. Parenter. Sci. Technol.*, 43 (1989) 231.
2 M. L. Bender and M. Komiyama, *Cyclodextrin Chemistry*, Springer-Verlag, Berlin, Heidelberg, New York, 1978.
3 W. Saenger, *Angew. Chem. Int. Ed. Engl.*, 19 (1980) 344.
4 J. Szejtli, *Pharm. Technol.*, 15(6) (1991) 36.
5 T. Beesley, *Advanced Separation Technologies*, Whippany, NJ, personal communication, April 4, 1990.
6 J. C. Linden and C. L. Lawhead, *J. Chromatogr.*, 105 (1975) 125.
7 K. Kainuma, T. Nakakuki and T. Ogawa, *J. Chromatogr.*, 212 (1981) 126.
8 L. A. Th. Verhaar and B. F. M. Kuster, *J. Chromatogr.*, 234 (1982) 57.
9 R. B. Meagher and A. Furst, *J. Chromatogr.*, 117 (1976) 211.
10 R. Schwarzenbach, *J. Chromatogr.*, 117 (1976) 206.
11 V. Kahle and K. Tesařík, *J. Chromatogr.*, 191 (1980) 121.
12 A. D. Jones, I. W. Burns, S. G. Sellings and J. A. Cox, *J. Chromatogr.*, 144 (1977) 169.
13 F. M. Rabel, A. G. Caputo and E. T. Butts, *J. Chromatogr.*, 126 (1976) 731.
14 P. Vrátný, J. Čoupek, S. Vozka and Z. Hostomská, *J. Chromatogr.*, 254 (1983) 143.
15 L. A. Th. Verhaar, B. F. M. Kuster and H. A. Claessens, *J. Chromatogr.*, 284 (1984) 1.
16 N. W. H. Cheetham and P. Sirimanne, *J. Chromatogr.*, 207 (1981) 439.
17 J. Haginaka, Y. Nishimura, J. Wakai, H. Yasuda, K. Koizumi and T. Nomura, *Anal. Biochem.*, 179 (1989) 336.
18 T. Takeuchi, M. Murayama and D. Ishii, *J. Chromatogr.*, 477 (1989) 147.
19 K. Aitzetmüller, *J. Chromatogr.*, 156 (1978) 354.
20 H. D. Scobell, K. M. Brobst and E. M. Steele, *Cereal Chem.*, 54 (1977) 905.
21 M. R. Ladisch, A. L. Huebner and G. T. Tsao, *J. Chromatogr.*, 147 (1978) 185.
22 L. E. Fitt, W. Hassler and D. E. Just, *J. Chromatogr.*, 187 (1980) 381.
23 H. D. Scobell and K. M. Brobst, *J. Chromatogr.*, 212 (1981) 51.
24 J. Bouchard, E. Chornet and R. P. Overend, *J. Agric. Food Chem.*, 36 (1988) 1188.
25 R. Oshima, N. Takai and J. Kumanotani, *J. Chromatogr.*, 192 (1980) 452.
26 J. Havlicek and O. Samuelson, *Carbohydr. Res.*, 22 (1972) 307.
27 O. Samuelson and J. Havlicek, *Anal. Chem.*, 47 (1975) 1854.
28 R. B. Kesler, *Anal. Chem.*, 39 (1967) 1417.
29 Z. Hostomská-Chytilová, O. Mikeš, P. Vrátný and M. Smrž, *J. Chromatogr.*, 235 (1982) 229.
30 L. A. Th. Verhaar and B. F. M. Kuster, *J. Chromatogr.*, 210 (1981) 279.
31 P. Jandera and J. Churáček, *J. Chromatogr.*, 98 (1974) 55.
32 K. Koizumi, Y. Kubota, T. Tanimoto and Y. Okada, *J. Chromatogr.*, 454 (1988) 303.
33 B. B. Wheals and P. C. White, *J. Chromatogr.*, 176 (1979) 421.
34 K. Koizumi, T. Utamura and Y. Kubota, *J. Chromatogr.*, 409 (1987) 396.