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Journal of Chromatography A, 793 (1998) 57–62

JOURNAL OF CHROMATOGRAPHY A

Determination of impurities in α-cyclodextrin by gradient high-performance liquid chromatography with pulsed amperometric detection

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Received 15 April 1997; received in revised form 1 August 1997; accepted 8 August 1997

Abstract

A gradient normal-phase high-performance liquid chromatography (NP-HPLC) method has been developed for the determination of impurities in α-cyclodextrin. The method is selective for six linear sugars and several late-eluting unidentified impurities, as well as for α-, β- and γ-cyclodextrins. The HPLC system utilizes mobile phases consisting of acetonitrile–water (A, 64:36; B, 35:65), a 5-μm YMC Polyamine II column (250×4.6 mm I.D.), and pulsed amperometric detection. Experiments were conducted to determine the optimal mobile phase concentrations, establish the precision of the method, and demonstrate linearity and determine relative response factors for select sugars. The method was applied to select lots of commercial-grade α-cyclodextrin from two different manufacturers. The method is novel in that it offers gradient elution of strongly-retained impurities at ambient column temperatures and pulsed amperometric detection. © 1998 Elsevier Science B.V.

Keywords: Cyclodextrins; Oligosaccharides

1. Introduction

Cyclodextrins (CDs) are α-1,4 linked cyclic oligosaccharides, and are the primary products resulting from the degradation of starch by the glucosyltransferase enzyme [1]. Essentially empty cavities of molecular size, CDs form inclusion complexes with a guest molecule when the guest either partially or completely enters the cavity. Complex formation is dependent on geometric and stereoelectrical considerations; no covalent bonds are formed [2]. Among other benefits, this guest–host interaction can improve the aqueous solubility and chemical stability of the guest compound [2–6]. CDs have been incorporated into foods and pharmaceuticals with increasing frequency in recent years. α-CD, which consists of six glucopyranose units, has been used to improve the room temperature stability of the active ingredient in a Pharmacia and Upjohn lyophilized drug candidate.

CDs are known to degrade to glucose and a series of acyclic maltosaccharides under certain conditions, such as partial acid hydrolysis [1]. Various maltosaccharides, both cyclic and acyclic, are also produced during the synthetic process [5]. α-CD is not currently utilized in any approved pharmaceutical products. In the present regulatory climate, a new excipient material is viewed no differently than a new active

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0021-9673/98/$19.00
PII S0021-9673(97)00852-2

ingredient. Therefore, a high-quality analytical method for the determination of potential α-CD degradation and process impurities was needed. Numerous high-performance liquid chromatography (HPLC) methods for the analysis of CDs have been described which utilize a variety of stationary phases (C_8, C_{18}, NH_2, ion-exchange, etc.) and modes of detection (refractive index, pulsed amperometric, polarimetric and others) [3–16]. However, these methods either are not designed to resolve the CDs from the associated linear sugars, lack the desired level of sensitivity, or are incapable of eluting strongly-retained compounds.

Various normal-phase partition methods which employ acetonitrile–water mobile phases and amino or amino–cyano columns have demonstrated considerable potential for the separation of cyclodextrins [5,7,10,14]. The YMC Polyamine II column is used for the method described here, chosen after investigating several columns from different manufacturers. The CDs (α, β and γ) and linear sugars (glucose to maltohexaose) are separated using isocratic conditions; several strongly-retained, unidentified impurities are then eluted by using a gradient to increase the water content of the mobile phase. Pulsed amperometric detection (PAD), which has been broadly applied to the detection of carbohydrates [6,11–13,15,17–22], is utilized because of its proven sensitivity and relative transparency to variations in mobile phase composition. Impurities are quantified in concentrated sample preparations versus α-CD external standards of known purity prepared at 5% of the sample concentration.

2. Experimental

2.1. Chemicals and reagents

α-CD was obtained from Aldrich (Milwaukee, WI, USA) and American Maize (Hammond, IN, USA). β-CD, γ-CD and the linear sugars were obtained from Aldrich. Maltotetraose was also obtained from Sigma (St. Louis, MO, USA). The acetonitrile was HPLC grade from EM Science (Gibbstown, NJ, USA), and the water was from a Milli-Q reagent water system from Millipore (Bedford, MA, USA). The 0.5 *M* NaOH post-column reagent was prepared with an Acculute standard volumetric concentrate from Anachemia (Rouses Point, NY, USA).

2.2. Apparatus

The HPLC/data collection system consisted of a 941A data interface (for transfer of data to a VAX computer) from PE Nelson (Cupertino, CA, USA), a GMP-2 gradient pump from Dionex (Sunnyvale, CA, USA), a Dionex PAD-2 pulsed amperometric detector, an Acuflow Series II pump (for post-column base delivery) from SSI (State College, PA, USA) and a Model 728 autosampler from Alcott (Oakbrook Parkway, GA, USA). The column used was a Polyamine II (250×4.6 mm I.D., 5 μm particle size) from YMC (Wilmington, NC, USA). Preliminary mobile phase optimization studies were conducted with an RI-4 differential refractometer from Varian (Walnut Creek, CA, USA), a Varian 9090 autosampler and a Model 2150 pump from LKB (Bromma, Sweden).

2.3. Method

A resolution mixture was made by dissolving each of the linear sugars (1–2 mg each), β- and γ-CDs (3 mg each) and α-CD (13 mg) in 15 ml of water. 15 ml of acetonitrile was then added. The resolution between the critical peak pair (α-CD and maltotetraose) was required to be ≥1.7 $\{R_s=[1.177\cdot(T_2-T_1)]/(W_1+W_2)$, where T_2 and T_1 are the retention times of maltotetraose and α-CD, respectively, and W_1 and W_2 are the widths of the two peaks at 50% peak height$\}$.

α-CD solutions ranging from 0.05 to 10% of the sample concentration were prepared for the linearity experiments. Other sugars were tested in a range from 0.05 to 5% of the sample concentration. Samples for the determination of precision were prepared at the 0.1% and 1% levels by spiking select sugars into 2.5 mg/ml solutions of α-CD. Standards were prepared by dissolving 1.25 mg of α-CD of known purity in 10.0 ml of acetonitrile–water (50:50). Samples were prepared for impurity determinations by dissolving 25 mg of α-CD in 5 ml of water, then diluting to 10.0 ml with acetonitrile.

A 25-μl injection volume was used for all experiments. The duration times and applied pulse po-

tentials for the PAD were: T_1: 720 ms, E_1: 0.00 V; T_2: 180 ms, E_2: 0.80 V; T_3: 360 ms, E_3: −0.60 V (potentials are reported versus a Ag/AgCl reference electrode). The PAD response time was set at 1 s, and the output range was set at 3000 nA/V.

The column was maintained at ambient temperature. The mobile phase flow-rate was 1.3 ml/min, while 0.5 *M* NaOH was delivered post-column at a rate of 0.5 ml/min. Mobile phase A consisted of acetonitrile–water (64:36) and mobile phase B of acetonitrile–water (35:65). The gradient program used was:

t (min)	A:B
0.0	100:0
19.0	100:0
31.0	72:28
43.0	72:28
43.1	100:0

10 min were allowed for column re-equilibration at the end of the program.

3. Results and discussion

The isocratic portion of the HPLC method provides near-baseline resolution ($R_s \geq 1.7$) of the components in the resolution mixture at ambient temperature (Fig. 1). α-CD and maltotetraose constitute the critical peak pair. The isocratic segment of the method is moderately sensitive to minor mobile phase adjustments (e.g., α-CD shifted from about 10 to 12 min, and maltohexaose from 18 to 23 min, in going from a 63% to a 65% acetonitrile mobile phase). Similar isocratic separations were previously reported by White et al. [5] and Koizumi et al. [23]; however, these methods lacked the ability to elute strongly-retained impurities with a reasonable *k′*.

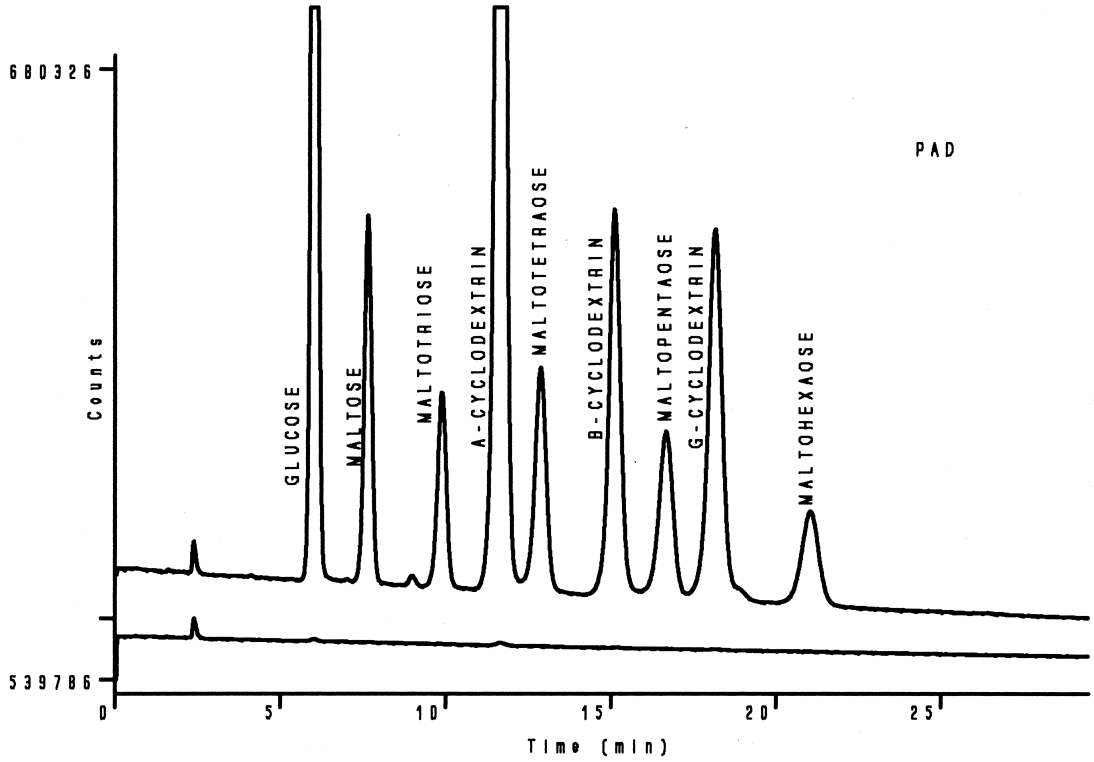

Fig. 1. Chromatograms of the resolution mixture containing α-CD and eight potential impurities (top) and blank injection (bottom) using a YMC Polyamine II column at ambient temperature, acetonitrile–water (64:36) mobile phase, and pulsed amperometric detection.

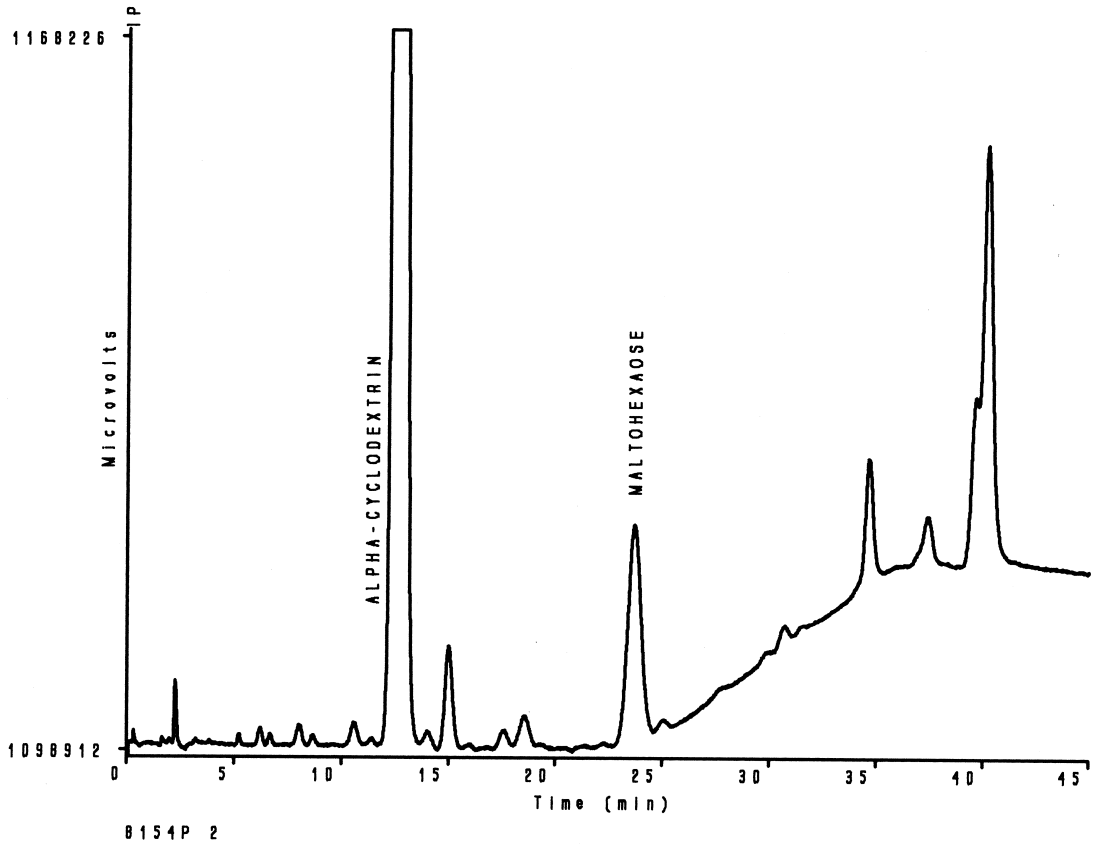

Fig. 2. Example chromatogram from α-CD Lot 2 generated using optimized gradient conditions.

The addition of a gradient to increase the water concentration of the mobile phase is effective for eluting such impurities (Fig. 2). Unlike refractive index detection, which has been extensively applied to CDs and other carbohydrates, PAD permits the use of a gradient because it is relatively insensitive to variations in mobile phase concentration.

Data which support the linearity of the method for select sugars are provided in Table 1. The linearity of an HPLC impurities method is often assessed over

Table 1
Summary of least-squares linear regression data for select sugars

	α-CD	β-CD	γ-CD	Glucose	Maltohexaose
No. of points	6	5	5	5	5
% Range	0.05–10	0.05–5	0.05–5	0.05–5	0.05–5
Slope[a]	$1.4711 \cdot 10^6$	$1.5380 \cdot 10^6$	$1.4846 \cdot 10^6$	$3.8494 \cdot 10^6$	$1.6241 \cdot 10^6$
Intercept[b]	32 158	−6573	−5207	7823	−4223
Correlation coefficient	0.9999	1.0000	1.0000	1.0000	1.0000
ESD[c] slope	9934	3151	1992	7132	2351
ESD[c] intercept	27 951	4037	2682	10 097	3017

[a] Units=μV s/μg.
[b] Units=μV s.
[c] ESD=Estimated standard deviation.

a broad range to provide assurance that impurities can be accurately quantified versus the major component peak. To compensate for the limited linear range which is characteristic of PAD, impurities in samples are quantified using α-CD external standards prepared at lower concentrations. The use of external standards enhances the overall sensitivity of the method by allowing impurities to be measured in concentrated samples where the α-CD response exceeds the linear range of the detector. However, it should be noted that sample concentrations are not so high as to require pre-heating to maintain CD solubility, as reported by others [5].

Waveform optimization experiments were conducted to ensure that the desired level of sensitivity could be achieved. Although LaCourse and Johnson [24] have described an elegant method for fully optimizing PAD waveforms using pulsed voltammetry, it was not practical to utilize this technique. Waveform optimization was instead conducted by fixing the pulse duration values and varying only the waveform potentials. For each set of potentials evaluated, the signal-to-noise ratio (S/N) was calculated for each of the three CDs. The waveform described in Section 2.3 provided the best S/N of those evaluated, and resulted in adequate method sensitivity. Limits of detection were higher (less sensitive) for the broader, late-eluting impurities with lower relative response factors. γ-CD, which elutes late in the chromatography and has the lowest relative response factor (Table 2), has a limit of detection of 0.005% (w/w) or 3 ng on-column ($S/N=3$, using an RMS noise measurement). Reliable measurements of individual impurities can be made at the 0.1% (w/w) level; relative standard deviations (R.S.D.s) of 3.5%, 4.7% and 9.2% were obtained for maltotriose, β-CD and maltohexaose, respectively ($n=6$ for each component). By comparison, the R.S.D.s at the 1% (w/w) level were 1.3%, 1.7% and 2.1% for maltotriose, β-CD and maltohexaose, respectively ($n=6$ for each component). Note that the rank follows the order of elution.

Table 2
Relative response factors, retention times and capacity factors for the CDs and linear sugars

Compound	RRF	Retention time (min)	k'
α-CD	1.00	11.6	3.8
β-CD	1.05	15.1	5.3
γ-CD	1.01	18.2	6.6
Glucose	2.62	6.0	1.5
Maltose	1.55	7.6	2.2
Maltotriose	1.27	9.9	3.1
Maltotetraose	1.16	12.9	4.4
Maltopentaose	1.11	16.7	6.0
Maltohexaose	1.10	21.1	7.8

Note: $t_0=2.4$ min (first significant baseline disturbance).

Impurities were measured in two α-CD lots, each from a different manufacturer. No impurities above 0.1% (w/w) were detected in Lot 1. Lot 2 contained 4.4% (w/w) total impurities, including 1.3% (w/w) maltohexaose and 2.0% (w/w) of a late-eluting unknown (Fig. 2). Unknown impurities were assumed to have a relative response factor of one.

Column ruggedness studies showed the Polyamine II column to be more durable than the other amine columns tested. However, the stability of the Polyamine II packing is not comparable to long-chain bonded-phase materials, such as C_{18} packings. Perhaps 150 injections can be made before the chromatography significantly degrades; column ruggedness data are still being gathered at this time. The use of a saturator column with similar packing material may increase the lifetime of the analytical column, although this has not been firmly established.

4. Conclusions

A HPLC method has been developed which uses isocratic conditions to separate six linear (glucose to maltohexaose) and two cyclic (β- and γ-CD) oligosaccharides in the presence of α-CD. Sample solutions do not require pre-heating to maintain CD solubility. Strongly-retained impurities are eluted by using a gradient to increase the water concentration in the mobile phase. Impurities are detected via pulsed amperometry, and are quantified in relatively concentrated sample preparations versus α-CD external standards prepared at 5% of the sample concentration. A detection limit of 0.005% (w/w) was calculated for γ-CD, the α-CD impurity with the highest detection limit. The precise measurement of individual impurities is possible at the 0.1% (w/w) level.

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