thermo scientific

APPLICATION NOTE 73011

HPAE-PAD determination of cyclodextrins

Authors

Manali Aggrawal and Jeffrey Rohrer

Thermo Fisher Scientific,
Sunnyvale, CA

Keywords

USP monograph, Betadex sulfobutyl ether sodium, α-cyclodextrin, β-cyclodextrin, γ-cyclodextrin, Dionex ICS 5000+ system, Dionex ICS 6000 system, excipient, USP-NF, Dionex CarboPac PA200 column

Goal

To evaluate the Thermo Scientific™ Dionex™ CarboPac™ PA200 column for the separation of α, β, and γ-cyclodextrins and for the determination of β-CD according to the USP Betadex Sulfobutyl Ether Sodium monograph.

Introduction

Cyclodextrins (CDs) are a family of cyclic oligosaccharides produced by enzymatic degradation of starch. The characteristic feature of these molecules is their ring-shaped, three-dimensional conical structure, with a hydrophilic outer surface and lipophilic cavity in the center capable of receiving a lipophilic "guest" molecule, provided its size and shape are compatible. Depending on the ring size, they are grouped into α-cyclodextrin (α-CD), a six-member sugar ring; β-cyclodextrin (β-CD), a seven-member sugar ring; and γ-cyclodextrin (γ-CD), an eight-member sugar ring. Their unique structural conformation together with versatile physicochemical features make them an ideal choice for drug delivery systems.[1,2] In the pharmaceutical industry, cyclodextrins have been used as complexing agents to increase water solubility of poorly soluble drugs and to increase their bioavailability and stability.[3-5] Worldwide, there are about 40 different pharmaceutical products containing cyclodextrins on the market.[6]

ThermoFisher SCIENTIFIC

Monographs for α-CD, β-CD, and γ-CD and two β-CD derivatives are in the European Pharmacopoeia[7] and the United States Pharmacopeia/National Formulary.[8-10] A monograph for CDs is also provided in the Handbook of Pharmaceutical Excipients, a compendial source, where CDs are classified as solubilizing and stabilizing agents.[11] β-CD is the most commonly used cyclodextrin. It can form inclusion complexes with a number of molecules of pharmaceutical interest. Betadex sulfobutyl ether sodium is a β-CD derivative with a sodium sulfonate salt separated from the lipophilic cavity by a sulfobutyl ether spacer group. It can act as a soluble carrier for drugs that have poor water solubility by formation of inclusion complexes. These advantageous properties led to betadex sulfobutyl ether sodium being included in the United States Pharmacopeia's (USP) National Formulary (NF).[10]

The presence of oxidizable hydroxyl groups makes CDs well suited for determination by high-performance anion-exchange (HPAE) chromatography in combination with pulsed amperometric detection (PAD). HPAE-PAD has been successfully used for the sensitive determination of CDs.[12-16] Various anion exchange columns have been used for these determinations including the Thermo Scientific™ Dionex™ IonPac™ AS6 Analytical and Guard Column (later renamed the Thermo Scientific™ Dionex™ CarboPac™ PA1 Analytical and Guard Columns),[12] Dionex CarboPac PA1,[15] and Thermo Scientific™ Dionex™ CarboPac™ PA100 Analytical and Guard Columns.[16]

The USP-NF Betadex Sulfobutyl Ether Sodium monograph describes an HPAE-PAD method for the determination of β-cyclodextrin impurity in betadex sulfobutyl ether sodium.[10] According to the monograph, betadex is separated on a Thermo Scientific™ Dionex™ IonPac™ AS11 Analytical & Guard Column followed by PAD detection using the specified three-potential waveform with a conventional gold working electrode (CWE). Application Note AN72779[17] demonstrated that the β-cyclodextrin could be successfully determined using the monograph conditions. Two different four-potential waveforms were also tested along with a disposable gold electrode on a PTFE substrate and these tests yielded comparable results. In this application note, the column is changed to a Thermo Scientific™ Dionex™ CarboPac™ PA200 Analytical and Guard Column. The Dionex CarboPac PA200 column is a nonporous, high-efficiency, polymeric anion-exchange column that provides the highest resolution available for oligosaccharide analysis by HPAE.[18] The Dionex CarboPac PA200 column includes smaller-particle-size packing material (5.5 µm) than is used in the Dionex IonPac AS11 column (13 µm). Separation is achieved on a Dionex CarboPac PA200 column followed by detection using the four-potential waveform with a gold disposable working electrode. Key performance parameters were evaluated and compared including separation, system suitability, linearity, limits of detection, and precision. Two samples were analyzed. The percentage of betadex results were compared with the acceptance criteria in the USP monograph. The application of the Dionex CarboPac PA200 column to separating other CDs was also examined.

Experimental

Equipment

- Thermo Scientific™ Dionex™ ICS-5000⁺ system including:
  - Thermo Scientific™ Dionex™ ICS-5000⁺ DP Dual Pump with degas option (P/N 079975)
  - Thermo Scientific™ Dionex™ ICS-5000⁺ DC Detector/ Chromatography Compartment (P/N 075943) with dual temperature zones, two injection valves
  - Thermo Scientific™ Dionex™ ICS-6000 ED Electrochemical Detector (P/N 072042) and Thermo Scientific™ Dionex™ ICS-6000 ED Electrochemical Detector Cell (P/N 072044)
  - Thermo Scientific™ Dionex™ ICS-6000 ED Electrochemical Detector Ag/AgCl pH Reference Electrode (P/N 061879)
  - Thermo Scientific™ Dionex™ Electrochemical Detector Gold on PTFE Disposable Electrodes, pack of 6 (two 2.0 mil gaskets included) (P/N 066480)
- Thermo Scientific™ Dionex™ AS-AP Autosampler (P/N 074925) with cooling tray option (recommended)
- Thermo Scientific™ Dionex™ AS-AP Autosampler Vial Kit, polystyrene, 10 mL, with caps and blue septa (P/N 074228) or Thermo Scientific™ Dionex™ AS-AP Autosampler Vial Kit, polypropylene, 1.5 mL, with caps and septa (P/N 079812)
- Thermo Scientific™ Nalgene™ Rapid-Flow™ Sterile Disposable Filter Units with Nylon Membrane, 0.2 µm filter units, 1000 mL, 90 mm diameter (P/N 164-0020)

Reagents and standards

- Deionized (DI) water, Type I reagent grade, 18 MΩ·cm resistance or better
- Sodium hydroxide 50% (w/w) (Fisher Scientific P/N SS254-500)
- Sodium nitrate, Acros Organics (Product code 424155000)
- α-Cyclodextrin, Sigma-Aldrich (Product code C4642)
- γ-Cyclodextrin, Sigma-Aldrich (Product code C4892)
- USP Betadex RS standard (USP, Part number 1154569)
- Sulfobutyl ether β-cyclodextrin sodium (Carbosynthe Product code OS15979)
- USP Betadex Sulfobutyl Ether Sodium RS (USP, Product code 1065550)

Preparation of solutions and reagents

Eluent A: 100 mM sodium hydroxide

To prepare 1 L of 100 mM sodium hydroxide, either pipette 5.2 mL or weigh 8 g 50% (w/w) sodium hydroxide into a plastic 1 L volumetric flask containing approximately 800 mL degassed DI water. If pipetting, use a plastic 5 or 10 mL sterile serological pipette with 0.1 mL gradations. Briefly stir this solution (15–30 s) and then bring to volume. Immediately transfer this solution to the plastic eluent bottle on the HPAE-PAD system and blanket it with helium or nitrogen at 34 to 55 kPa (5 to 8 psi). Please refer to TN 71[19] for details on eluent preparation for HPAE-PAD.

Eluent B: 100 mM sodium hydroxide / 0.5 m sodium nitrate

To prepare 1 L of 100 mM NaOH/0.5 M sodium nitrate, first dissolve 42.5 g of high-purity anhydrous sodium nitrate into approximately 800 mL DI water. Vacuum filter this solution through a 0.2 µm nylon filter to remove particles from the sodium nitrate that can damage parts of the pump. This filtration is often slow, as the insolubles in the sodium nitrate will gradually clog the filter. Remember to disconnect the vacuum from the solution before turning off the vacuum pump to prevent backwash into the filtered eluent. After filtration, transfer the solution to a plastic 1 L volumetric flask, add 5.2 mL or 8 g of 50% NaOH (for 100 mM), and bring to volume. Immediately transfer this solution to the plastic eluent bottle on the HPAE-PAD system and blanket it with helium or nitrogen at 34 to 55 kPa (5 to 8 psi).

Standard preparation

Accurately weigh 50 mg of USP β-cyclodextrin RS standard in a 50 mL volumetric flask and add DI water up to the mark to make 1000 mg/L stock standard. Sonicate and mix well for 1 min. Using 25 mL volumetric flasks dilute the stock standard 10× and then dilute that solution 50× to obtain a final concentration of 100 mg/L and 2 mg/L, respectively. Dilute the 100 mg/L stock solution appropriately to prepare the following calibration standards: 0.5, 1, 2.5, 5, 7.5, and 10 mg/L.

Samples

Two samples were analyzed with the limit of betadex test in the USP Betadex Sulfobutyl Ether Sodium monograph:

- Sample A: Sulfobutyl ether β-cyclodextrin sodium (Carbosynthe Product code OS15979)
- Sample B: USP Betadex Sulfobutyl Ether Sodium RS (USP, Product code 1065550)

Sample preparation

Accurately weigh 20 mg of sample in a 10 mL volumetric flask and add DI water up to the mark to make a 2000 mg/L solution. Mix well.

To prepare 1 ppm spiked sample, weigh 20 mg of sample in a 10 mL volumetric flask and add 100 µL of 100 mg/L β-cyclodextrin RS standard, then bring to volume with DI water. Mix well.

To prepare 2 ppm spiked sample, weigh 20 mg of sample in a 10 mL volumetric flask and add 200 µL of 100 mg/L β-cyclodextrin RS standard, then bring to volume with DI water. Mix well.

System preparation and setup

Prepare the Dionex ICS-5000$^+$ system by adding the Dionex ICS-5000$^+$ SP/DP Pump module and ICS-5000$^+$ DC Detector/Chromatography module in the Thermo Scientific™ Chromeleon™ Instrument Configuration

manager. Prime the pump with the new eluent following the step-by-step instructions in the Dionex ICS-5000+ Ion Chromatography System Operator's Manual.[20] After priming the pump, condition the column using the eluent at 0.5 mL/min for 30 min. Then connect the column to the electrochemical detector. Prepare the electrochemical cell by rinsing the cell body, the well of the reference electrode, and the inlet tube thoroughly with DI water and dry with an absorbent tissue. Caution: Do not touch the working electrode gold surface with any paper products as this can contaminate the working electrode. Assemble the cell following the Dionex ICS 5000+ operator's manual[20] and Dionex ED User's Compendium for Electrochemical Detection[21] by first installing the working electrode gasket flat against cell body. Avoid any wrinkles in the gasket, as this will cause a poor fit and subsequent leaks and poor detection. Install the disposable working electrode and spacer block. Install the yoke block by squeezing the tabs and sliding it on the cell body. Align the yoke block parallel to the cell body and rotate the yoke block knob clockwise until you hear a click. Install the cell into the ED module and connect the yellow cable to the yellow port. To calibrate the pH-Ag/AgCl reference electrode, install the reference electrode blue cable into the black port. Immerse the reference electrode in pH 7 buffer to at least mid-level of the electrode. Select the "pH Calibration" button on the ED Panel and follow the instructions to calibrate the electrode using pH 10 buffer. After calibration is complete, rinse the buffer solution off the electrode with DI water, and gently, but firmly, screw in the reference electrode clockwise into the reference electrode port of the electrochemical cell until the reference electrode is finger-tight. For best results, replace the reference electrode after six months of use. While running the ED cell, bubbles may be trapped in the cell. Air bubbles in the cell can cause spikes in the baseline. To prevent air from becoming trapped in the cell, increase the backpressure on the cell by connecting backpressure tubing to the cell outlet. The backpressure limit for the ED cell is 690 kPa (100 psi). Do not exceed this limit. Six feet of black (0.01 in. i.d.) PEEK tubing at the cell outlet should generate 30–40 psi backpressure, which can prevent bubble formation.

Table 1. Conditions

Columns:	Dionex CarboPac PA200, Analytical, 3 × 250 mm (P/N 062896) Dionex CarboPac PA200, Guard, 3 × 50 mm (P/N 062895)		
Eluent A:	100 mM Sodium hydroxide		
Eluent B:	100 mM Sodium hydroxide, 0.5 M Sodium nitrate		
	Time (min)	*Eluent A (%)*	*Eluent B (%)*
Gradient program (For separation of α-CD, β-CD, and γ-CD):	0	98	2
	2	98	2
	18	94	6
	18	98	2
	20	98	2
	Time (min)	*Eluent A (%)*	*Eluent B (%)*
Gradient program (For limit of betadex):	0	92	8
	5	92	8
	5	0	100
	10	0	100
	10	92	8
	20	92	8
Flow rate:	0.5 mL/min		
Column temp.:	25 °C		
Injection volume:	10 µL		
Detection:	Pulsed Amperometry		
	Time (s)	*Potential (V)*	*Integration*
Waveform 1 (TN21)[22]:	0.00	+0.10	
	0.20	+0.10	Begin
	0.40	+0.10	End
	0.41	-2.0	
	0.42	-2.0	
	0.43	+0.6	
	0.44	-0.1	
	0.50	-0.1	
Working electrode:	Gold disposable, with 2 mil. gasket		
Reference electrode:	Ag/AgCl		
Run time:	20 min		

Results and discussion

Separation of α, β, and γ-cyclodextrin

The common anion exchange chromatography columns used for the analysis of CDs as described in the literature are the Dionex IonPac AS6,[12] Dionex CarboPac PA1,[15] and Dionex CarboPac PA100[16] columns. Here, the Dionex CarboPac PA200 column was evaluated for the separation and determination of CDs. The Dionex CarboPac PA200 column was developed to provide high-resolution separations of charged and neutral oligosaccharides and is the recommended column for these applications. To obtain a fast separation of CDs with a good resolution, various eluent combinations were tested. Through the optimization of gradient conditions, the best gradient program was selected (Table 1). The separation was designed to be completed within 20 min. A linear gradient of sodium nitrate (10–30 mM) was applied to separate the three cyclodextrins.

Figure 1 shows the chromatogram of a mixture of α-CD, β-CD, and γ-CD. In general, retention of a homologous series of carbohydrates on this column increases as the degree of polymerization (DP) increases. Thus, the elution order of cyclodextrins is α-CD (DP=6), β-CD (DP=7), and γ-CD (DP=8). Over the course of the analysis, the retention time (RT) relative standard deviations of the three CDs (n = 6) ranged from 0.2% to 0.4%.

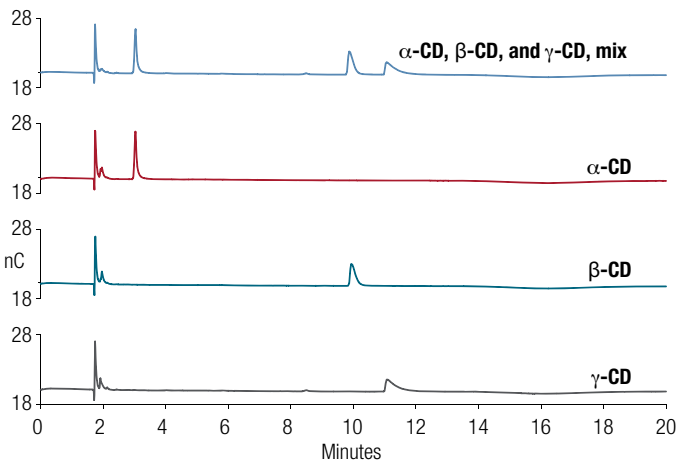

Figure 1. Cyclodextrin chromatography (2 mg/L)

The peak shapes of α-CD and β-CD were good, but the γ-CD peak showed tailing, similar to published separations.[12] The eluents in the published separations were 150 mM sodium hydroxide solution containing 140–300 mM sodium acetate as a modifier to elute cyclodextrins. Nitrate was used instead of acetate as a pushing agent. Nitrate has been shown previously[23] to be a stronger pushing agent and yields better resolution of higher polymers. Nitrate eluent has been used for the limit of betadex test, which is discussed in more detail in the following section.

Limit of betadex test according to USP monograph

Separation

The USP monograph for betadex sulfobutyl ether sodium describes a limit of betadex test based on isocratic separation with sodium nitrate using a Dionex IonPac AS11 anion-exchange column and alkaline mobile phase (pH = 12.4) followed by PAD using a specified three-potential waveform and a conventional gold working electrode. A Dionex CarboPac PA200 column was evaluated for this test using the conditions listed in Table 1. As mentioned above, sodium nitrate was used as a pushing agent instead of sodium acetate. With acetate as the pushing agent, a slight decrease in retention time (RT) of β-cyclodextrin with multiple injections was observed for betadex samples (data not shown). This loss in RT was not observed using nitrate eluent. This is most likely due to the incomplete removal of strongly retained analytes (betadex sulfobutyl ether sodium in this case) from the column.[10,17]

Figure 2 displays a chromatogram of a 2 mg/L USP β-cyclodextrin RS standard solution. The RT for β-cyclodextrin is 2.50 min. The chromatography conditions are isocratic followed by a step-change to a higher eluent concentration. In the first five minutes the analytes of interest are eluted using 40 mM sodium nitrate/100 mM sodium hydroxide. At 5 min a column clean-up/regeneration step is initiated using 500 mM sodium nitrate/100 mM sodium hydroxide. This step is to remove the strongly retained analytes from the column. The separation is followed by PAD using a four-potential waveform on a gold disposable working electrode. In comparison to the Dionex IonPac AS11 column, β-CD is better retained on the Dionex CarboPac PA200 column. The peak shape/asymmetry is comparable to that on the Dionex IonPac AS11 column. Figure 3 shows the comparison of chromatograms of 2 mg/L USP β-cyclodextrin RS standard solution on a Dionex IonPac AS11 column and Dionex CarboPac PA 200 column. Table 2 lists the comparison of the parameters such as RT, peak shape, symmetry, etc. on these two columns.

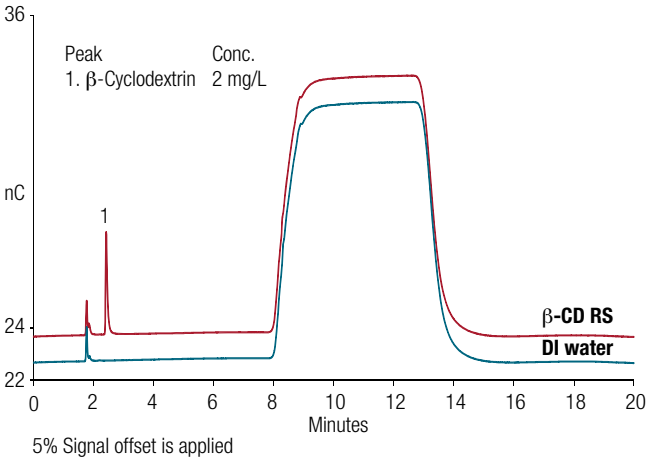

Figure 2. Chromatogram of 2 mg/L β-cyclodextrin RS standard

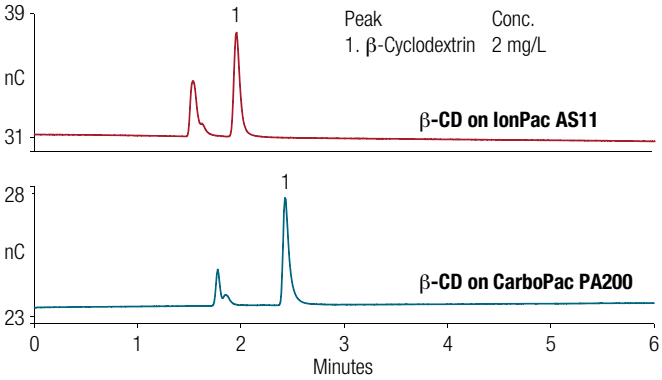

Figure 3. Comparison of chromatograms of 2 mg/L USP β-cyclodextrin RS standard solution on a Dionex IonPac AS11 column and a Dionex CarboPac PA200 column

Table 2. Comparison of chromatographic parameters on two columns

	Dionex IonPac AS11 column (4 × 250 mm)	Dionex CarboPac PA 200 (3 × 250 mm)
Gradient	0-4 min: 25 mM NaOH, 5-10 min: 1 M $NaNO_3$/250 mM NaOH, 11-20 min: 25 mM NaOH	0-5 min: 40 mM $NaNO_3$/100 mM NaOH, 5-10 min: 0.5 M $NaNO_3$/100 mM NaOH, 10-20 min: 40 mM $NaNO_3$/100 mM NaOH
Retention time (min)	1.88	2.5 min
Peak area (nC·min)	0.459	0.282
Peak height (nC)	6.95	3.90
Peak asymmetry (EP)	1.65	1.80
Theoretical plates (EP)	5800	10,500

System suitability

In the USP monograph for betadex sulfobutyl ether sodium, the following system suitability requirement is specified: the relative standard deviation (RSD) is not more than 5% (area of β-cyclodextrin peak) for replicate injections of the standard solution. The RSD of the retention time, peak area, and peak height were determined for six replicate injections of the USP β-cyclodextrin RS standard solution. The RSDs were <0.2%, 0.6%, and 0.5%, respectively, for the betadex peak. Table 3 shows the comparison of two columns in terms of the USP system suitability criterion and other parameters.

Table 3. Comparison of USP system suitability on two columns

Column	Electrode	Waveform	Description	Peak area RSD (n=6)	Retention time RSD (n=6)	Peak height RSD (n=6)
AS11	CWE*	As specified in monograph	3-potential	0.49	0.22	0.43
AS11	DE**	Waveform 1	4-potential, 0.5 s	0.42	0.01	0.60
PA200	DE	Waveform 1	4-potential, 0.5 s	0.61	0.15	0.50

*CWE- Conventional Working Electrode
**DE- Disposable Electrode

Linearity, and limits of detection (LOD) and quantitation (LOQ)

Method linearity was evaluated by constructing calibration curves of six concentrations of USP β-cyclodextrin RS standard ranging from 0.5 mg/L to 10 mg/L. The calibration plot (Figure 4) shows the data is best fit using a quadratic equation and that yielded a coefficient of determination (r^2) greater than 0.9999.

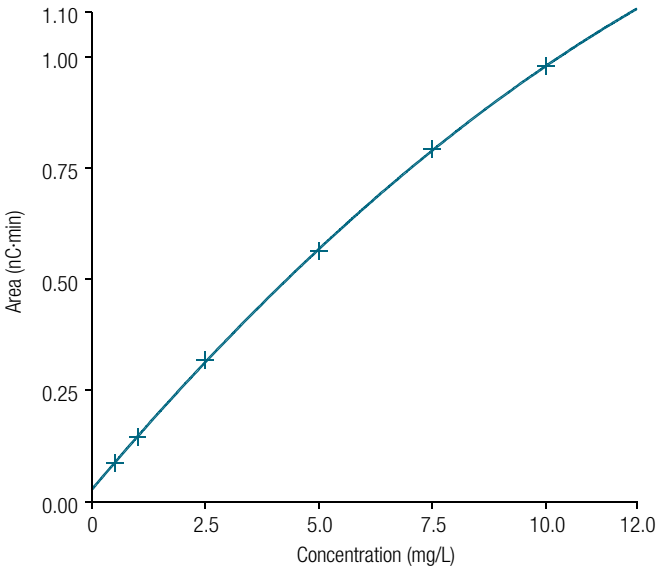

Figure 4. Calibration curve of β-cyclodextrin RS

To determine the LOD and LOQ, the baseline noise was first determined by measuring the peak-to-peak noise in a representative one-minute segment of the baseline where no peaks elute, but close to the β-cyclodextrin peak. The signal was determined from the average peak height of three injections of 0.1 mg/L USP β-cyclodextrin RS standard. The LOD and LOQ were determined by 3× and 10× S/N, respectively. The estimated LOD and LOQ were 0.03 and 0.1 mg/L, respectively. Table 4 lists the comparison of S/N, LODs, and LOQs on two columns.

Sample analysis

Two commercial betadex sulfobutyl ether samples (Table 5) were purchased and tested using the eluent conditions listed in Table 2 with the Dionex CarboPac PA200 column. Figure 5 displays the chromatograms of the two samples. As noted earlier, betadex elutes at ~2.5 min and the method concludes with a step change to a strong eluent. This step change allows the strongly retained analytes to come off the column. For both the samples, a cluster of peaks at ~8.5 min were observed. These are likely from betadex sulfobutyl ether sodium.

Table 4. Comparison of S/N, LOD, and LOQ on two columns

Column	Electrode	Waveform	Signal (S) (nC)	Noise (N) (nC)	S/N	LOD (mg/L)	LOQ (mg/L)
AS11	CWE*	As specified in monograph	1.30	0.067	19.4	0.015	0.052
AS11	DE**	Waveform 1	0.32	0.045	7.16	0.042	0.140
PA200	DE	Waveform 1	0.15	0.016	9.63	0.030	0.100

*CWE- Conventional Working Electrode
**DE- Disposable Electrode

Table 5. Limit of β-cyclodextrin

Sample	Betadex Sulfobutyl Ether Sample	USP Limit (%)	Measured (%)
A	Carbosynthe, Product code OS15979	<0.1	0.002
B	USP, Product code 1065550	<0.1	0.046

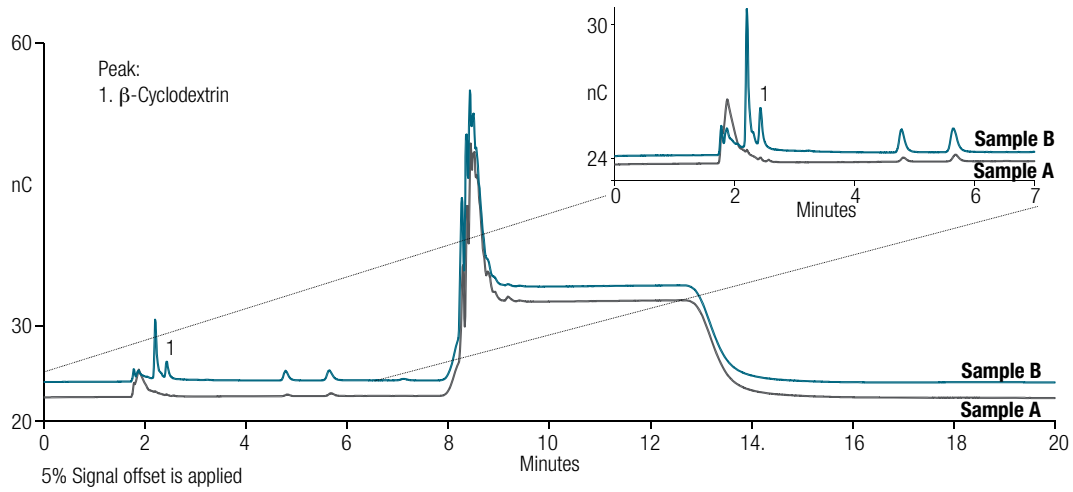

Figure 5. Chromatograms of Samples A and B (2000 mg/L)

It is evident from the response in both chromatograms that Sample A contains significantly less β-cyclodextrin impurity than Sample B. The percentage of β-cyclodextrin in samples is calculated as specified in the USP monograph:

$$\text{Percentage} = (r_U / r_S) \times (C_S / C_U) \times F \times 100$$

Where:

r_U = Peak area response for beta cyclodextrin from the Sample solution

r_S = Peak area response for beta cyclodextrin from the Standard solution

C_S = Concentration of USP β-Cyclodextrin RS in the standard solution (µg/mL)

C_U = Concentration of Betadex Sulfobutyl Ether Sodium in the Sample solution (mg/mL)

F = Conversion factor (10-3 mg/µg)

The USP acceptance criterion for the β-cyclodextrin content in betadex sulfobutyl ether sodium is that the product should contain less than 0.1% β-cyclodextrin. Results are listed in Table 5 and both samples pass the limit test.

Method ruggedness and accuracy

Method ruggedness was evaluated by measuring the response of β-cyclodextrin standards and samples under the same conditions but on two separate columns. The RTs of β-cyclodextrin on two columns were found to differ by ~3 %; i.e. 2.425 min on Column 1 and 2.500 min on Column 2. The USP monograph system suitability (NMT 5%) and acceptance criteria (NMT 0.1%) were met by both columns.

Method accuracy was evaluated by measuring recoveries of USP β-cyclodextrin RS standard spiked into sample at two concentrations. The recovery percentages were calculated according to formula given below:

$$\textit{Recovery \%} = \frac{\textit{(C spiked sample-C unspiked sample)}}{\textit{(C analyte added)}} \cdot 100$$

Figure 6 shows the representative chromatogram of unspiked and spiked Sample A. The recovery percentages for β-cyclodextrin standard at two spiked levels in both samples are in the range of 90% to 110%.

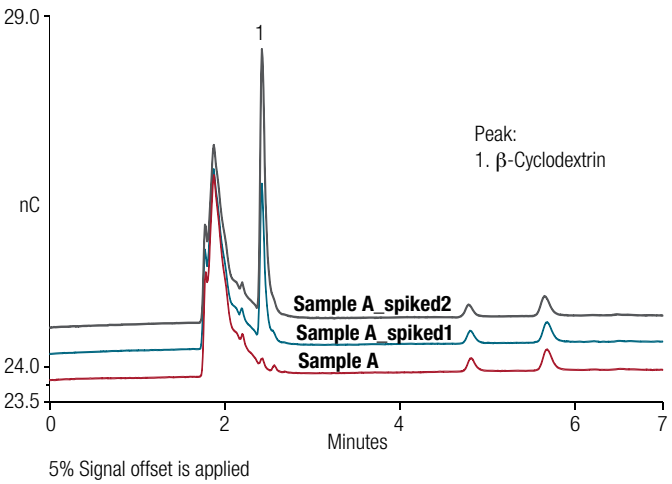

Figure 6. Chromatograms of Sample A and Spiked Sample A with 1 and 2 mg/L β-cyclodextrin RS standard

thermo scientific

Conclusion

In this application note an HPAE-PAD method was successfully developed using a Dionex CarboPac PA200 column for the separation and determinations of α-, β-, and γ-CDs. The limit of betadex test in the USP Betadex Sulfobutyl Ether Sodium monograph could be successfully performed using the Dionex CarboPac PA200 column. Two commercial betadex sulfobutyl ether sodium samples were tested and found to contain betadex impurity under the specified limit prescribed in the USP monograph. The separation, linearity, reproducibility, and sensitivity were found to meet or exceed the current USP Betadex Sulfobutyl Ether Sodium monograph performance requirements.

References

1. Vyas, A.; Saraf, S. Cyclodextrin based novel drug delivery systems, *J. Incl. Phenom. Macrocycl. Chem.* **2008,** *62,* 23–42.
2. Challa, A. T.; Ahuja, J.; Ali, R.; Khar, K. Cyclodextrins in Drug Delivery: An Updated Review, *AAPS PharmSciTech,* **2005,** *6,* E329–E357.
3. Brewster, M. E.; Loftsson, T. Cyclodextrins as pharmaceutical solubilizers, *Advanced Drug Delivery Reviews.* **2007,** *59,* 645–666.
4. Arima, H.; Motoyama, K.; Higashi, T. Potential use of cyclodextrins as drug carriers and active pharmaceutical ingredients. *Chem. Pharm. Bull.* **2017,** *65,* 341–348.
5. Hu, Q.-D.; Tang, G.-P.; Chu, P.K. Cyclodextrin-based host–guest supramolecular nanoparticles for delivery: From design to applications. *Acc. Chem. Res.* **2014,** *47,* 2017–2025.
6. Cyclolab. Approved Pharmaceutical Products Containing Cyclodextrins. Available online: http://www.pharmacompass.com/pAssets/pdf/party/Zibo-Qianhui-Biological-Technology-Co-Ltd-regulatory-1435833292.pdf (accessed on 20 April 2019).
7. COE/EDQM (2008). The European Pharmacopoeia 6.0. Council of Europe European (COE)–European Directorate for the Quality of Medicines (EDQM).
8. USP (2007). The United States Pharmacopeia 30. Port City Press, Baltimore, pp. 1056, 1071.
9. Gamma- Cyclodextrin, The United States Pharmacopeia, National Formulary 30th, United States Pharmacopeial Convention, Rockville MD, 2012, 1776–1778.
10. Betadex Sulfobutyl Ether Sodium, The United States Pharmacopeia 41st ed., National Formulary 36th, United States Pharmacopeial Convention, Rockville MD, 2018, 5224.
11. Rowe, R.J., Sheskey, P.J., Owen, S.C. Handbook of Pharmaceutical Excipients, 5th ed., Pharmaceutical Press, London, and American Pharmacists Association, Washington, DC, **2006**. 217.
12. Koizumi, K.; Kubota, Y.; Tanimoto, T.; Okada, Y. Determination of cyclic glucans by anion-exchange chromatography with pulsed amperometric detection. *J. Chromatogr.* **1988,** *454*, 303–310.
13. Haginaka, J.; Nishimura, Y.; Wakai, J.; Yasuda, H.; Koizumi, K.; Nomura, T. Determination of cyclodextrins and branched cyclodextrins by reversed-phase chromatography with pulsed amperometric detection and a membrane reactor. *Anal. Biochem.* **1989,** *179,* 336.
14. Fukuda, M.; Kubota, Y.; Ikuta, A.; Hasegawa, K.; Koizimi, K. Microanalyses of β-Cyclodextrin and Glucosyl-β-cyclodextrin in Biological Matrices by High-Performance Liquid Chromatography with Pulsed Amperometric Detection. *Anal. Biochem.* **1993,** *212,* 289.
15. Koo, Y.S.; Lee, H.W.; Jeon, H.Y.; Choi, H.J.; Choung, W.J.; and Shim, J.H. Development and characterization of cyclodextrin glucanotransferase as a maltoheptaose-producing enzyme using site-directed mutagenesis. *Protein Engineering, Design & Selection,* **2015,** *28*(11), 531–537.
16. Dura, A.; Yokoyama, W.; Rosell, C.M. Effects of Cyclodextrin Glycosiltranferase Modified Starch and Alfa and Beta Cyclodextrins on Plasma Glucose and Lipids Metabolism in Mice. *J Drug Des Res.* **2017,** *4*(5), 1051.
17. Thermo Fisher Scientific. Application Note AN72779: Limit of β-cyclodextrin (Betadex) in Betadex Sulfobutyl Ether Sodium [Online: https://appslab.thermofisher.com/App/4177/determination-betadex (accessed March 2019).
18. Thermo Scientific Dionex CarboPac PA 200 column product manual. https://www.thermofisher.com/order/catalog/product/062896 (accessed March 2019).
19. Thermo Fisher Scientific. Technical Note TN71: Eluent Preparation for High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection. [Online: http://tools.thermofisher.com/content/sfs/brochures/TN-71-Eluent-Preparation-for-High-Performance-Anion-Exchange-Chromatogarphy-with%20APD-TN-70669.pdf (accessed March 2019).
20. Thermo Scientific Dionex ICS-5000+ Ion Chromatography System Installation Instructions. Document No. 065343. Revision 05 December 2011. https://tools.thermofisher.com/content/sfs/manuals/87165-Man-IC-ICS5000-Install-Dec2011-DOC065343-05.pdf (accessed March 2019).
21. Thermo Scientific Electrochemical Detection User's Compendium, P/N 065340-02, April 2013. https://assets.thermofisher.com/TFS-Assets/CMD/manuals/Man-065340-Electrochemical-Detection-Man065340-EN.pdf (accessed March 2019).
22. Thermo Fisher Scientific. Technical Note TN21: Optimal Settings for Pulsed Amperometric Detection of Carbohydrates Using the Dionex ED40 Electrochemical Detector. http://dionex.su/en-us/webdocs/5050-TN-21-Optimal-Settings-PAD-Carbohydrates-ED40-TN70670-EN.pdf (accessed March 2019).
23. Thermo Fisher Scientific. Application Note AN1013: Polysialic Acid Analysis: Separating Polymers with High Degrees of Polymerization. https://appslab.thermofisher.com/App/1583/an1013-polysialic-acid-analysis-separating-polymers-with-high-degrees-polymerization (accessed March 2019).

Find out more at **www.thermofisher.com/chromatography**

ThermoFisher SCIENTIFIC