

Limit of β -cyclodextrin (betadex) in betadex sulfobutyl ether sodium

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Keywords

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Goal

To evaluate the limit test for β -cyclodextrin in the USP Betadex Sulfobutyl Ether Sodium monograph and to evaluate the same limit test with the 4-potential waveform recommended for carbohydrate detection and a disposable working electrode

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. They are chemically and physically stable macromolecules with a number of hydrogen donors and acceptors, making them hydrophilic, and thus they typically do not permeate lipophilic membranes. Their unique structural conformation together with versatile physicochemical features makes them an ideal choice for drug delivery systems. 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. Depending on the ring size, they are grouped into

α -cyclodextrin: six-member sugar ring, β -cyclodextrin: seven-member sugar ring, and γ -cyclodextrin: eight-member sugar ring. Betadex (β -cyclodextrin) is made of homogeneous α -1,4-linked α -D glucopyranose units in a seven-member ring. Betadex sulfobutyl ether sodium is a β -cyclodextrin derivative with a sodium sulfonate salt separated from the lipophilic cavity by a sulfobutyl ether spacer group. This molecule can act as a soluble carrier for drugs that have poor water solubility by forming inclusion complexes.¹⁻³ These advantageous properties led to betadex sulfobutyl ether sodium being included in the United States Pharmacopeia (USP) National Formulary (NF).⁴

Due to the absence of a chromophore or fluorophore in their chemical structure, direct detection of CDs has been limited to techniques such as refractive index (RI), evaporative light scattering (ELSD), pulsed amperometry (PAD), or mass spectrometry (MS). Generally RI and ELSD are low-sensitivity detection techniques, and therefore can only be used for the analysis of relatively high concentrations of CDs.⁴⁻⁶ For low CD concentrations, PAD and MS detection methods have been successfully applied.^{7,8} The presence of oxidizable hydroxyl groups on these cyclic oligosaccharides, makes them 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 analysis of CDs.⁹⁻¹¹ The USP-NF Betadex Sulfobutyl Ether Sodium monograph describes a HPAE-PAD method for the determination of β -cyclodextrin impurity in betadex sulfobutyl ether sodium.⁴

In this application note, we evaluated the USP monograph method. Betadex was separated on a Thermo Scientific™ Dionex™ IonPac™ AS11 column followed by PAD detection using the specified 3-potential waveform with a gold conventional working electrode (CWE). The method was further evaluated with the 4-potential waveform recommended for carbohydrates and a gold disposable working electrode (DE). In this additional evaluation only the waveform was changed compared to the monograph method. Key performance parameters were evaluated 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.

Experimental

Equipment

- Thermo Scientific™ Dionex™ ICS-5000+ HPIC™ system including:
 - DP dual pump module (P/N 079975) with degas option
 - DC standard bore detector compartment (P/N 075943) with dual temperature zones, two injection valves
 - Electrochemical detector (P/N 072042) and cell (P/N 072044)
 - pH-Ag/AgCl reference electrode (P/N 061879)
 - Carbohydrate disposable Au working electrode, pack of six (two 2.0 mil gaskets included) (P/N 066480)
 - Gold electrode with gasket and polishing kit (P/N 079850)
- Thermo Scientific™ Dionex™ AS-AP Autosampler
- Thermo Scientific™ Dionex™ Vial Kit, 10 mL Polystyrene with Caps and Blue Septa; P/N 074228
- Thermo Scientific™ Nalgene™ Rapid-Flow™ 0.2 μ m filter units, 1000 mL, nylon membrane, 90 mm diameter (P/N 164-0020)

Reagents and standards

- Deionized (DI) water, Type I reagent grade, 18 M Ω -cm resistance or better
- Sodium hydroxide 50% (w/w) (Fisher Scientific P/N SS254-500)
- Potassium nitrate (Acros Organics™ product code 424155000)
- Betadex RS standard (USP product code 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: 25 mM sodium hydroxide

To prepare 1 L of 25 mM sodium hydroxide, either pipette 1.3 mL or weigh 2.0 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).

Eluent B: 250 mM sodium hydroxide / 1M potassium nitrate

To prepare 1 L of 250 mM NaOH/1M potassium nitrate, first dissolve 101.10 g of high-purity anhydrous potassium nitrate into approximately 800 mL DI water. Vacuum filter this solution through a 0.2 µm nylon filter to remove particles from the potassium nitrate that can damage parts of the pump. This filtration is often slow, as the insolubles in the potassium 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 13 mL or 20.0 g of 50% NaOH (for 250 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). Please refer to TN 71¹³ for details on eluent preparation for HPAE-PAD.

Standard preparation

Accurately weigh 50 mg of USP β-cyclodextrin RS standard in a 25 mL volumetric flask and add DI water up to the mark to make 2000 mg/L stock standard. Sonicate and mix well for 1 min. Using 25 mL volumetric flasks, dilute the stock standard 100× and then dilute that solution 10× to obtain final concentrations of 20 mg/L and 2 mg/L, respectively. Dilute the 20 mg/L stock solution appropriately to prepare the following calibration standards: 0.4, 0.8, 1.6, 2.5, and 3.5 mg/L.

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.

To prepare 0.5 ppm spiked sample, weigh 20 mg of sample in a 10 mL volumetric flask and add 250 µL of 20 mg/L β-cyclodextrin RS standard, then bring to volume with DI water.

To prepare 1 ppm spiked sample, weigh 20 mg of sample in a 10 mL volumetric flask and add 500 µL of 20 mg/L β-cyclodextrin RS standard, then bring to volume with DI water.

System preparation and setup

Prepare the Dionex ICS-5000+ IC system by adding the Dionex ICS-5000+ SP/DP Pump module, Dionex ICS-5000+ DC Detector/Chromatography module in the 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.¹⁴ After priming the pump, condition the column using the eluent at 1 mL/min for 30 min. Then, connect the column to the electrochemical detector. Prepare the electrochemical cell by rinsing the cell body, working electrode, and gasket thoroughly with DI water and dry with a lab wipe. Assemble the cell following the Dionex ICS 5000+ Ion Chromatography System Operator's Manual¹⁴ and Dionex ED User's Compendium for Electrochemical Detection¹⁵ 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 conventional working electrode with the metal face down over the gasket. 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 including using pH 10 buffer. After calibration is complete, rinse the buffer solution off the electrode with DI water, and gently, but firmly, screw in or rotate 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.

Conditions (USP monograph method)

System:	Dionex ICS-5000+ HPIC system		
Columns:	Dionex IonPac AS11, Analytical, 4 × 250 mm (P/N 044076) Dionex IonPac AG11, Guard, 4 × 50 mm (P/N 044078)		
Eluent A:	25 mM sodium hydroxide		
Eluent B:	250 mM sodium hydroxide, 1 M potassium nitrate		
Gradient Program:	<i>Time (min)</i>	<i>Eluent A (%)</i>	<i>Eluent B (%)</i>
	0	100	0
	4	100	0
	5	0	100
	10	0	100
	11	100	0
	20	100	0
Flow Rate	1 mL/min		
Column Temp.:	50 °C		
Injection Volume:	20 µL		
Detection:	Pulsed Amperometry		
Waveform 1:	<i>Time (s)</i>	<i>Voltage (V)</i>	
	0.00	0.1	
	0.30	0.1, Start Integration	
	0.50	0.1	
	0.50	0.1, Stop Integration	
	0.51	0.60	
	0.59	0.60	
	0.60	-0.60	
	0.65	-0.60	
Working Electrode:	Gold CWE		
Reference Electrode:	Ag/AgCl		
Run Time:	20 min		

Results and discussion

The USP monograph for betadex sulfobutyl ether sodium describes a limit of betadex test based on isocratic separation using an anion exchange column and alkaline mobile phase (pH = 12.4) followed by PAD.

Chromatography

The USP Betadex Sulfobutyl Ether Sodium monograph describes a 25 × 0.4 cm analytical anion-exchange column containing a L61 packing column type for the separation of β-cyclodextrin and sulfobutyl ether β-cyclodextrin. This is a description of the Dionex IonPac AS11, Analytical, 4 × 250 mm column. The Dionex IonPac AS11 column is a hydroxide-selective, strong-anion-exchange column consisting of ethylvinylbenzene cross-linked with 55% divinylbenzene and latexed with smaller particles containing alkanol quaternary ammonium ions. Figure 1 displays the chromatographic profile of 2 mg/L USP β-cyclodextrin RS standard solution using the chromatography conditions of the USP monograph method. The retention time (RT) for β-cyclodextrin is ~1.98 min. According to the USP Pharmacopial Forum (PF) publication, betadex elutes at approximately 2 min.¹⁶ The specified chromatography conditions are isocratic followed by a step change to a higher eluent concentration. In the first four minutes the analytes of interest are eluted using Eluent A (25 mM NaOH). After t=4 min a column clean-up/regeneration step is initiated using Eluent B (250 mM sodium hydroxide with 1M potassium nitrate). We believe this step is present to remove the betadex sulfobutyl ether sodium from the column. The separation is followed by PAD using a 3-potential waveform on a gold CWE.

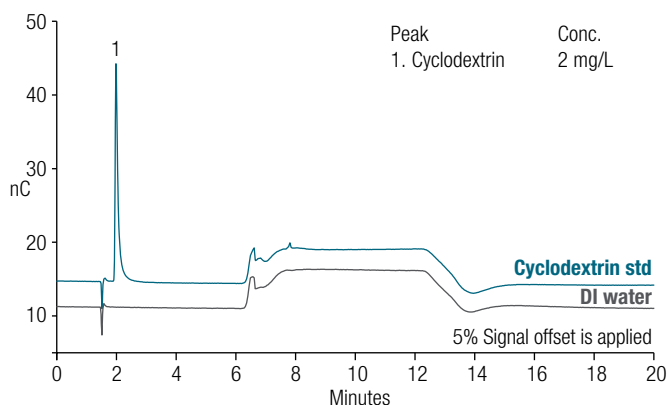


Figure 1. Chromatograms of DI water and 2 mg/L β-cyclodextrin RS standard using USP method conditions

The 3-potential waveform (Figure 2) uses the following three potentials: E1 set to a small positive value 0.1 V (versus the Ag/AgCl reference) for sample oxidation/detection, E2 at 0.6 V limit for oxidative cleaning of the working electrode, E3 at -0.6 V to reduce gold oxide to gold and prepare the electrode for detection.

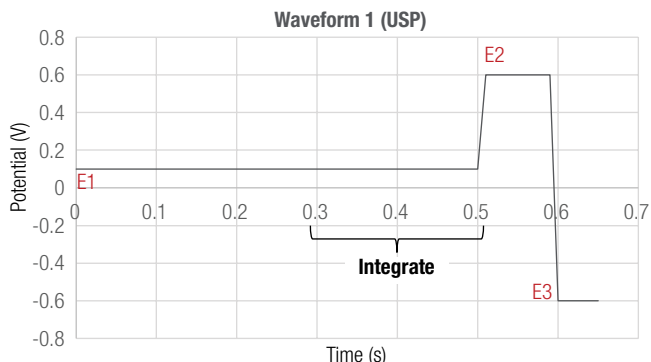


Figure 2. Schematics of the 3-potential waveform used for detection of β -cyclodextrin

System suitability

In the USP monograph for betadex sulfobutyl ether sodium the following system suitability requirement is specified as relative standard deviation (RSD) 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 (Figure 3). The RSDs were <0.2%, 0.5%, and 0.5%, respectively for the betadex peak.

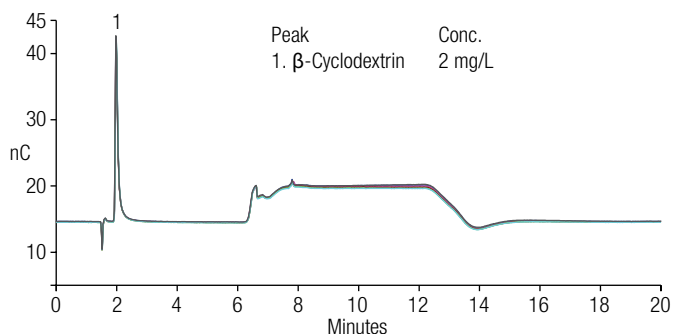


Figure 3. Overlay of six injections of 2 mg/L β -cyclodextrin RS standard

Method ruggedness, linearity, and limits of detection (LOD) and quantitation (LOQ)

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 ~15%; i.e. 1.68 min on Column 1 and 1.98 min on Column 2 (Figure 4). The USP monograph system suitability (NMT 5%) and acceptance criteria (NMT 0.1%) were met by both columns, but the LOD was found to be slightly lower on Column 2 compared to Column 1. This is due the fact that resolution of the betadex peak from the other peaks near the void is better on Column 2 than that on Column 1.

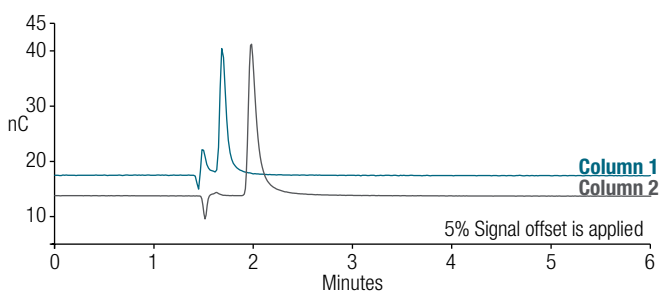


Figure 4. Chromatograms of 2 mg/L β -cyclodextrin RS standard on each of two columns

Method linearity was evaluated by constructing calibration curves of five concentrations of USP β -cyclodextrin RS standard ranging from 0.4 mg/L to 3.5 mg/L. The calibration plot of peak area versus concentration was fit using linear regression and that yielded a coefficient of determination (r^2) greater than 0.999.

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 \times and 10 \times S/N, respectively. The estimated LOD and LOQ were 0.015 and 0.052 mg/L, respectively.

Sample analysis

Two commercial betadex sulfobutyl ether samples (Table 1) were purchased and tested under USP monograph conditions. Figure 5 displays the chromatograms of the two samples. As mentioned

Table 1. Limit of β -cyclodextrin

Sample	Betadex Sulfobutyl Ether Sample	USP Limit (%)	Measured (%)
A	Carbosynthe, Product code OS15979	< 0.1	Not detected
B	USP, Product code 1065550	< 0.1	0.066

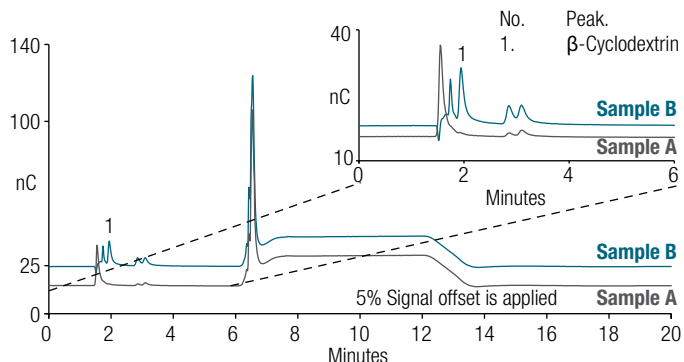


Figure 5. Chromatograms of Samples A and B (2000 mg/L)

previously, betadex elutes at ~2 min under isocratic conditions of Eluent A (25 mM NaOH) followed by a step change at 4 min from 100% Eluent A to 100% Eluent B (250 mM sodium hydroxide / 1 M potassium nitrate). This step change allows the strongly retained analytes to elute from the column. For both the samples, a large peak at ~6.5 min was observed. We believe this peak is betadex sulfobutyl ether sodium.

It is evident from the response in both chromatograms that Sample A contains significantly less β -cyclodextrin impurity than Sample B. In fact the amount of betadex impurity in Sample A is less than the limit of detection. The percentage of β -cyclodextrin in Sample B 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 ($\mu\text{g}/\text{mL}$)

C_U = Concentration of betadex sulfobutyl ether sodium in the sample solution (mg/mL)

F = Conversion factor (10^{-3} mg/ μg)

The USP acceptance criteria 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 1 and both samples pass the limit test.

Sample recovery

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

$$\text{Recovery \%} = \frac{C_{\text{spiked sample}} - C_{\text{unspiked sample}}}{C_{\text{analyte added}}} \times 100$$

Figure 6 shows the representative chromatograms of unspiked and spiked Sample A at 0.5 mg/L and 1.0 mg/L β -cyclodextrin standard. The recovery percentages for β -cyclodextrin standard at two spiked levels in both samples are in the range of 90–110%.

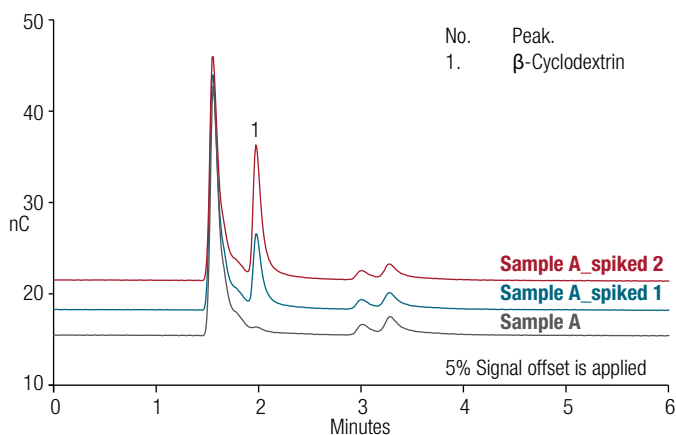


Figure 6. Chromatograms of Sample A and Spiked Sample A with 0.5 and 1 mg/L β -cyclodextrin RS standard

Comparison of waveforms

3-potential vs 4-potential waveform

Detection of β -cyclodextrin was also evaluated with two different 4-potential waveforms. Figure 7 displays the schematics of the 4-potential waveforms (Waveform 2 and 3) used in this study. Waveforms 2 and 3 differ from Waveform 1 (3-potential waveform used in USP monograph method) in that they use a negative rather than a positive potential for electrode cleaning. When positive cleaning potentials are used there is a gradual decrease in carbohydrate peak areas over time due to working electrode wear (recession below the plastic housing). Four-potential waveforms do not promote electrode recession. This difference is demonstrated and discussed in detail in Thermo Scientific Technical Note 21.¹⁷ One of the greatest advantages of using negative potential cleaning in the 4-potential waveforms is consistent long term peak area response. Another benefit is that both 4-potential waveforms require less time, either 500 ms or 330 ms, and therefore data can be collected with greater frequency. This results in more data points per peak, which is important for achieving better peak area accuracy and reproducibility for chromatographically efficient early eluting peaks like β -cyclodextrin. The response, however, is generally lower compared to initial use with the 3-potential waveform. The 4-potential waveforms also have a greater sensitivity to dissolved oxygen due to a higher background compared to the 3-potential waveform. Figure 8 displays the chromatographic profiles of a 2 mg/L of β -cyclodextrin RS standard run using the three different waveforms. As discussed above, the signal response of β -cyclodextrin with Waveform 1 is higher than Waveforms 2 and 3. The signal response for the 0.33 s 4-potential waveform (Waveform 3) is almost half compared to the 0.5 s waveform (Waveform 2). This is due to the fact that integration time of the 500 ms waveform is more than the integration time for the 330 ms waveform. The advantage is more data points and thus sharper peaks, which is beneficial for an early eluting peak like β -cyclodextrin.

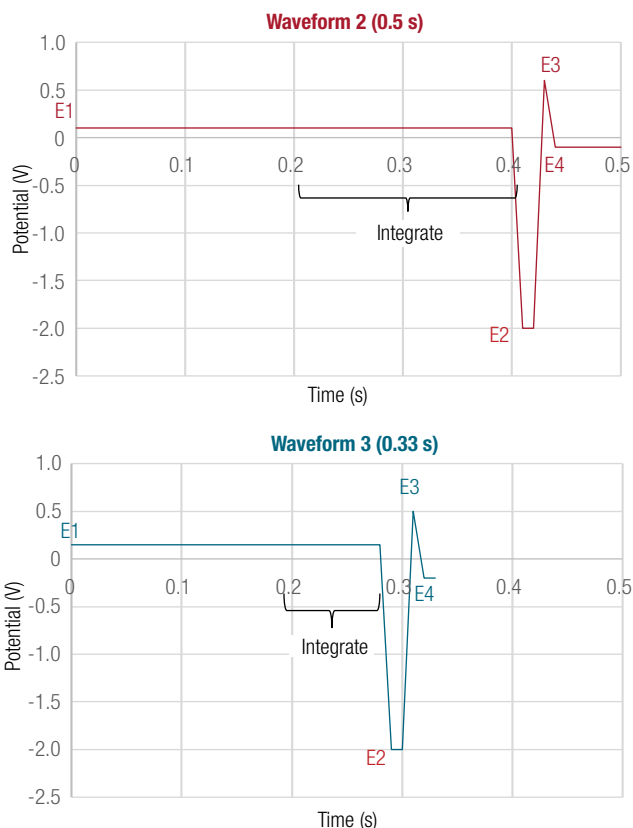


Figure 7. Schematics of 4-potential waveforms used for detection of β -cyclodextrin

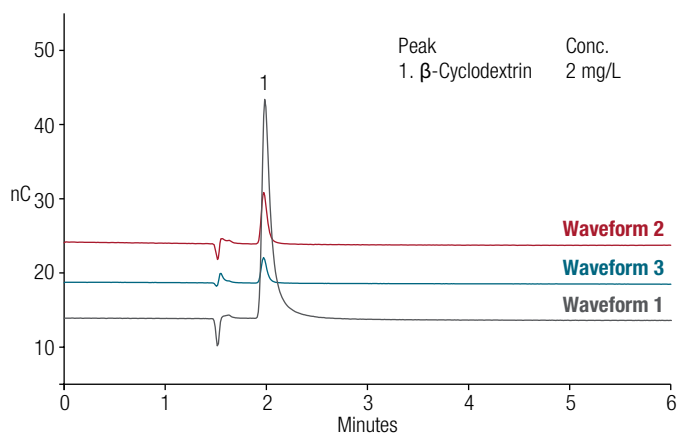


Figure 8. Chromatogram of β -cyclodextrin RS standard using a conventional gold working electrode and three waveforms

We further evaluated 4-potential waveforms (Waveforms 2 and 3) with gold on PTFE disposable working electrodes. The use of a disposable working electrode simplifies system maintenance as they do not require electrode polishing. These working electrodes give consistent response and last for at least four weeks. Figure 9 displays the comparison of the two types of working electrode, conventional and disposable with two 4-potential waveforms. The signal response is similar with both conventional and disposable working electrodes. The background current is slightly higher with the disposable working electrode, but S/N, LOD, and LOQ are similar for the two electrodes (Table 2). The limit of detection with 3-potential waveforms is lower in comparison to 4-potential waveforms, but both the

4-potential waveforms pass the USP monograph system suitability (Table 3) and USP limit of betadex acceptance criterion (Table 4).

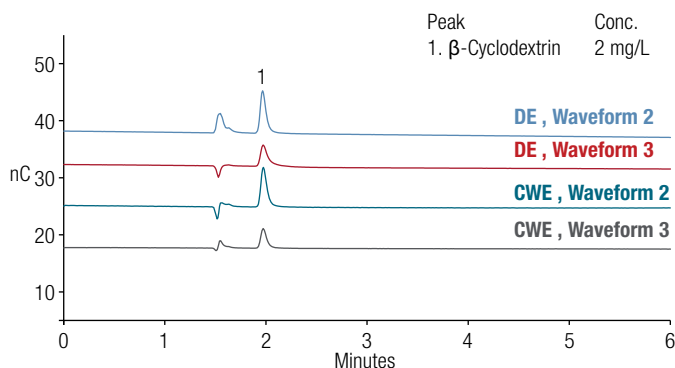


Figure 9. Chromatograms of β -cyclodextrin RS standard run with different waveforms and electrodes

Table 2. Comparison of S/N, LOD, and LOQ for different waveforms

Electrode	Waveform	Signal (S) (nC)	Noise (N) (nC)	S/N	LOD (mg/L)	LOQ (mg/L)
CWE	3-potential (USP method)	1.30	0.067	19.4	0.015	0.052
	4-potential, 0.5 s	0.280	0.057	4.91	0.061	0.204
	4-potential, 0.33 s	0.131	0.029	4.52	0.066	0.221
DE	4-potential, 0.5 s	0.322	0.045	7.16	0.042	0.140
	4-potential, 0.33 s	0.163	0.029	5.62	0.053	0.178

Table 3. Results of method precision for different waveforms used in this study

Electrode	Waveform	Peak Area RSD % (n=6)	Retention Time RSD % (n=6)	Peak Height RSD % (n=6)
CWE	3-potential (USP method)	0.49	0.22	0.43
	4-potential, 0.5 s	0.78	0.01	1.15
	4-potential, 0.33 s	0.56	0.01	0.62
DE	4-potential, 0.5 s	0.42	0.01	0.60
	4-potential, 0.33 s	0.69	0.16	0.69

Table 4. Comparison of waveforms (USP acceptance criterion)

Electrode	Waveform	β -Cyclodextrin RS standard		Sample B		USP Limit
		Amount μ g/mL	Peak area	Amount mg/mL	Peak area	Result NMT 0.1%
CWE	3-potential (USP method)	1.995	2.999	2.000	1.999	0.066
	4-potential, 0.5 s	1.994	0.399	2.000	0.261	0.065
	4-potential, 0.33 s	1.983	0.189	2.000	0.127	0.066
DE	4-potential, 0.5 s	2.027	0.459	2.000	0.297	0.066
	4-potential, 0.33 s	2.044	0.237	2.000	0.150	0.064

Conclusion

In this application note, we demonstrated that the determination of β -cyclodextrin could be successfully performed using the USP Betadex Sulfobutyl Ether Sodium monograph conditions. Two commercial betadex sulfobutyl ether sodium samples were tested and found to contain betadex impurity under the specified limit prescribed in the USP monograph. We also demonstrated that this method could be executed with two different 4-potential waveforms and a disposable gold electrode on a PTFE substrate with comparable results. The separation, linearity, reproducibility, and sensitivity were found to meet or exceed the current USP Betadex Sulfobutyl Ether Sodium monograph performance requirements.

References

- Vyas, A.; Saraf, S.; Saraf, S. Cyclodextrin based novel drug delivery systems, *J. Incl. Phenom. Macrocycl. Chem.*, **2008**, *62*, 23–42.
- Challa, T.; Ahuja, A.; Ali, J.; Khar, R.K. Cyclodextrins in Drug Delivery: An Updated Review, *AAPS PharmSciTech*, **2005**, *6*, E329-E357.
- Brewster, M. E.; Loftsson, T. Cyclodextrins as pharmaceutical solubilizers, *Advanced Drug Delivery Reviews*, **2007**, *59*, 645–666.
- Betadex Sulfobutyl Ether Sodium, The United States Pharmacopeia 41st ed., National Formulary 36th, United States Pharmacopeial Convention, Rockville MD, 2018, 5224.
- Kinalekar, M.S.; Kulkarni, S.R.; Vavia, P.R. Simultaneous determination of alpha, beta and gamma cyclodextrins by LC. *J. Pharm. Biomed. Anal.* **2000**, *22*, 661.
- White, G.; Katona, T. Zodda, J.P.; Eakins, M.N. Determination of the impurity profile of γ -cyclodextrin by high-performance liquid chromatography. *J. Chromatogr.* **1992**, *625*, 157.
- Agüeros, M.; Campanero, M.A.; Irache, J.M. Simultaneous quantification of different cyclodextrins and Gantrez by HPLC with evaporating light scattering detection. *J. Pharm. Biomed. Anal.* **2005**, *39*, 495–502.
- Hammes, W.; Bourscheidt, C.; Büchler, U.; Stodt, G.; Bökens, H. Quantitative determination of α -cyclodextrin in human plasma by liquid chromatography/positive ion electrospray mass spectrometry. *J. Mass Spectrom.* **2000**, *35*, 378.
- Blum, W.; Aichholz, R.; Ramstein, P.; Fetz, A.; Raschdorf, F. Determination of 2-hydroxypropyl-gamma-cyclodextrin in plasma of cynomolgus monkeys after oral administration by gas chromatography-mass spectrometry. *J. Chromatogr. B* **1998**, *720*, 171.
- Fukuda, M.; Kubota, Y.; Ikuta, A.; Hasegawa, K.; Koizumi, 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.
- 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.
- 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.
- Thermo Scientific Technical Note 71: Eluent Preparation for High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection. [Online] <https://assets.thermofisher.com/TFS-Assets/CMD/Technical-Notes/tn-71-hpae-pad-eluent-prep-tn70669-en.pdf> (accessed July 9, 2018).
- Thermo Scientific Dionex ICS-5000 Ion Chromatography System Installation Instructions. Document No. 065343. Revision 05 December 2011 [Online] <https://assets.thermofisher.com/TFS-Assets/CMD/manuals/87165-Man-IC-ICS5000-Install-Dec2011-DOC065343-05.pdf> (accessed July 9, 2018).
- Thermo Scientific Electrochemical Detection User's Compendium, P/N 065340-02, April 2013. [Online] <https://assets.thermofisher.com/TFS-Assets/CMD/manuals/Man-065340-Electrochemical-Detection-Man065340-EN.pdf> (accessed July 9, 2018).
- Betadex Sulfobutyl Ether Sodium, The United States Pharmacopeia., Pharmacopeial Forum (PF) 36(2) In-Process Revision, United States Pharmacopeial Convention, Rockville MD.
- Thermo Scientific Technical Note 21: Optimal Settings for Pulsed Amperometric Detection of Carbohydrates Using the Dionex ED40 Electrochemical Detector [Online] <https://assets.thermofisher.com/TFS-Assets/CMD/Application-Notes/TN-21-Optimal-Settings-Pulsed-Amperometric-Detection-Carbohydrates-ED40-TN70670-EN.pdf> (accessed July 9, 2018).

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