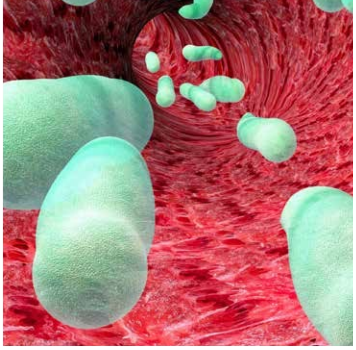
Application Note

Drugs & Pharmaceuticals



The most reliable LC-EC applications for Drugs & Pharmaceuticals analysis

Aminoglycoside drugs
Amikacin
Framycetin Sulphate
Gentamicin Sulphate
Kanamycin Sulphate
Lincomycin
Neomycin
Netilmicin
Spectinomycin
Tobramycin

Macrolide antibiotics
Azithromycin
Azaerythromycin
Clarithromycin
Erythromycin
Roxithromycin

Antipsychotic drugs
Clozapine
Olanzapine
Risperidone

PET imaging tracer
Fluorodeoxyglucose (FDG)
FDG impurities

Pharmaceuticals, API
Acetaminophen
Artemether
Artemisinin
Dihydro- artemisinin
Etoposide
Epinephrine
Heparin
mesna BNP7787
8-OH-DPAT
Vincristine

Betadex Sulfobutyl Ether Sodium According to USP method

- **ALEXYS HPAEC-PAD Analyzer**
- **Flow cell with Au working electrode and Ag reference electrode**
- **Meets method description US Pharmacopoeia 38-NF 36 (2015)**
- **Reproducible and robust**

Summary

The contents of β-cyclodextrin in sulfobutyl ether β-cyclo-dextrin sodium was analyzed using the exact method and conditions described in the official 2015 USP monograph [8]. In this application note typical results obtained with the ALEXYS® HPAEC-PAD analyzer are reported, demonstrating its performance for the impurity analysis of sulfobutyl ether β-cyclodextrin bulk material.

Introduction

Cyclodextrins (CD's) are a group of cyclic oligosaccharides produced from starch by bacterial digestion. The characteristic feature of these molecules is their ring-shaped, three-dimensional conical structure, with a hydrophobic cavity in the center, which is capable of receiving a lipophilic "guest" molecule, provided its size and shape are compatible [1]. β-cyclodextrin (cycloheptaamylose) is made of homogeneous cyclic (α-1,4-linked) α-D- glucopyranose units in a seven member ring.

Sulfobutyl ether β-cyclodextrin sodium (also called Betadex sulfobutyl ether sodium) is a chemically modified β-cyclodextrin used as drug delivery system. It can act as a soluble carrier for drugs that have poor water solubility, by formation of inclusion complexes [2-4]. Since CD's do not contain chromophores or fluorophores, their direct detection by optical techniques, like refractive index (RI) and evaporative light scattering (ELSD) lack sensitivity and can only be used when analyzing relatively large concentrations of CD's. However, due to the presence of oxidizable hydroxyl groups on these cyclic oligosaccharides, High Pressure Anion-Exchange Chromatography in combination with Pulsed Amperometric Detection (HPAEC-PAD) can be successfully utilized for the sensitive analysis of CD's [5-7].

The United States Pharmacopoeia (USP) describes a compendial method for the impurity analysis of β-cyclodextrin in Sulfobutyl Ether β-cyclodextrin Sodium using HPAEC-PAD [8].

Method

The USP (38-NF33) method for Sulfobutyl ether β-cyclodextrin sodium is based on isocratic separation using an anion exchange column and alkaline mobile phase (pH = 12.4) followed by PAD.

Separation

In the monograph the use of the following column type is described for the separation of β-cyclodextrin and Sulfobutyl ether β-cyclodextrin: '*size 250 x 4 mm ID analytical anion-ex-*

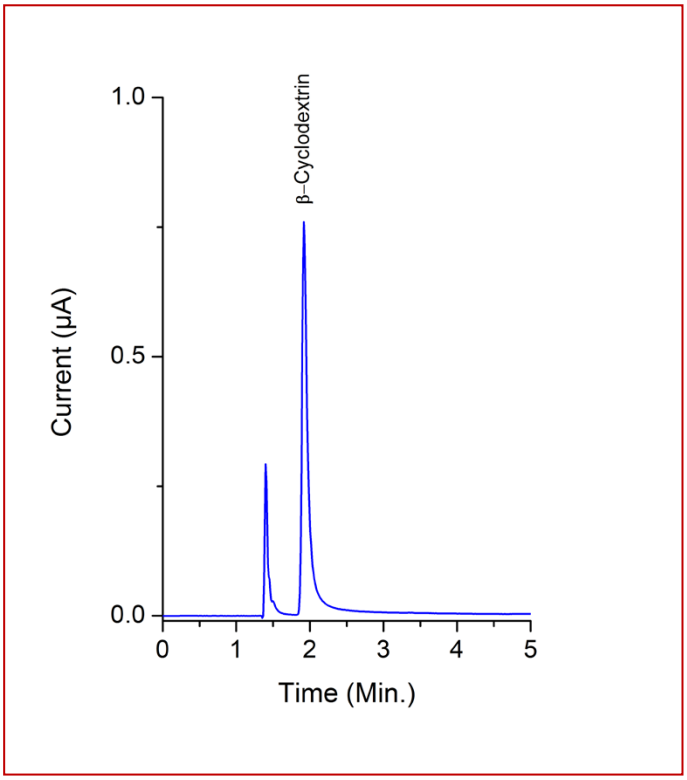
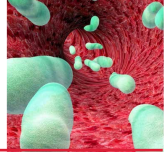


Figure 1: Chromatogram obtained from a 20 µL injection of a 2 µg/mL β-cyclodextrin RS standard in water.

Table 1

Conditions	
LC system	ALEXYS HPAEC-PAD Analyzer
Columns	IonPac™ AS11, 50 x 4 mm ID + 250 x 4 mm ID (USP column packing L61) All columns: Thermo Scientific™ Dionex™
Mobile phase (MP)	MP A: 25mM sodium hydroxide MP B: 250mM sodium hydroxide, 1M potassium nitrate
Flow rate	1.0 mL/min
Injection	20 µL
Temperature	50 °C for separation and detection
Flow cell	FlexCell* with Au WE, stainless steel AE and Ag/AgCl RE, 50 µm spacer
Potential waveform (3-step)	E1, E2, E3: +0.1, +0.6, -0.6 V ts, t1, t2, t3: 0.2, 0.5, 0.1, 0.05 s
I-cell	about 0.2 µA
ADF	0.5 Hz
Range	2 µA/V

* Original work done with 3 mm Au VT-03, stainless steel AE and Ag/AgCl RE, 50 µm spacer



change column containing a L61 packing, which is defined as a hydroxide-selective, strong anion-exchange resin consisting of a highly cross-linked core of 13 µm microporous particles, pore size less than 10 Å, and consisting of ethylvinylbenzene cross-linked with 55 % divinylbenzene with a latex coating composed of 85 nm diameter microbeads bonded with alkanol quaternary ammonium ions (6 %).'

An anion exchange column filled with 'L61' packing and a matching guard column were used for the method evaluation (Table 1).

The separation is based on a step-gradient profile as specified in the USP monograph (Table 2). In the first 4 minutes of the run the analytes of interest are eluted isocratically using a mobile phase of 25 mM NaOH. After t=4 min a column clean-up/ regeneration step is initiated using a mobile phase consisting of 250mM sodium hydroxide with 1M potassium nitrate.

For the tests shown in this note the ALEXYS HPAEC-PAD Analyzer was equipped with two pumps to enable high pressure binary gradient elution for the column clean-up step.

Table 2

Gradient profile

Time (min)	*Mobile phase A (%)*	*Mobile phase B (%)*
0	100	0
4	100	0
5	0	100
10	0	100
11	100	1
20	100	0

Mobile phase preparation

The eluents were carefully prepared manually using a commercial 50% NaOH solution, carbonate-free. The diluent was deionized water (resistivity >18 MΩ-cm), which was first sonicated and then sparged with Helium 5.0 prior to use. The appropriate amount of NaOH was carefully pipetted into the diluent to minimize the introduction of carbonate in the solution. The bottles with mobile phase and column clean-up solution were blanketed with Helium during the analysis to minimize the build-up of carbonate ions in the mobile phase and thus assure a reproducible analysis.

Detection

For the detection of β-cyclodextrins using PAD the monograph specifies the use of a flow cell with an Au working electrode (WE) and Ag reference electrode (RE). A 3-step potential waveform is used as described in the USP monograph (Table 1). The cell current was typically about 0.2 µA with these PAD settings under the applied conditions. The temperature for separation and detection was set to 50°C. Note that the DECADE Elite heater has an excellent temperature precision of < ± 0.05 °C, which lays well within the USP temperature stability requirement of 50 ± 2 °C.

Standards, samples and preparation

Standards: 10 mg of USP β-cyclodextrin RS standard (USP, part number 1154569) was accurately weighted and dissolved in 5 mL of water in a volumetric flask (sonicated for 1 minute and mixed). The obtained solution was subsequently 100x and 10x diluted using 25 mL volumetric flasks to obtain a final concentration of 2 µg/mL. The standard solution was also used as system suitability standard to check the USP system performance criteria.

Samples: 10 mg of sample was accurately weighted and dissolved in 5 mL of water in a volumetric flask (sonicated for 1 minute and mixed) to obtain a final concentration of 2 mg/mL. Two commercially available samples were prepared in this way:

(1) Sulfobutyl ether β-cyclodextrin sodium (Carbosynth, product code OS15979)

(2) USP Betadex Sulfobutyl Ether Sodium RS (USP, product code 1065550)

The samples are respectively abbreviated as sample OS15979 and 1065550 from this point onward.

Results

Figure 1 shows an example chromatogram from the 2 µg/mL USP β-cyclodextrin RS standard solution. The retention time for the β-cyclodextrin peak was 1.92 min. Note that only the relevant part of the chromatogram (first 5 minutes) containing the β-cyclodextrin peak is shown, and not the response during the column clean-up step from t= 5 - 20 minutes.

System suitability test

The USP monograph for Betadex sulfobutyl ether sodium specifies a system suitability requirement for the Relative Standard Deviation (RSD): it should not be more than 5% (area of β-cyclodextrin peak) for 6 replicate injections of the standard solution. The RSD requirement was therefore evaluated by analyzing 6 repetitive injections of the USP standard solution. The result shows that the system suitability requirement was met (Table 3).

Table 3

USP system suitability requirements

Parameter	*USP criteria*	*Measured*
$RSD_{Peak\ Area}$, β-cyclodextrin (n=6)	< 5%	0.3

Linearity, repeatability & LOD

The linearity for β-cyclodextrin was investigated in the concentration range of 0.4 µg/mL – 3.5 µg/mL. In this concentration range the correlation coefficient for peak area was better than 0.999. The relative standard deviation (RSD) for β-cyclodextrin was <0.1% for retention time, 0.3% for peak area and 0.7% for peak height based on 6 replicate injections of the USP β-cyclodextrin RS standard solution. The Limit of Detection (LOD) for β-cyclodextrin, calculated as the analyte response corresponding to 3x the ASTM noise (average peak-to-peak baseline noise of 30 segments of 0.5 min), was about 2.5 ng/mL.

Sample analysis

Two commercial β-Cyclodextrin sulfobutyl ether sodium salt samples were analyzed to make example chromatograms (Fig. 3 and 4). It is evident from the response in both chromatograms that sample OS15979 contains significantly

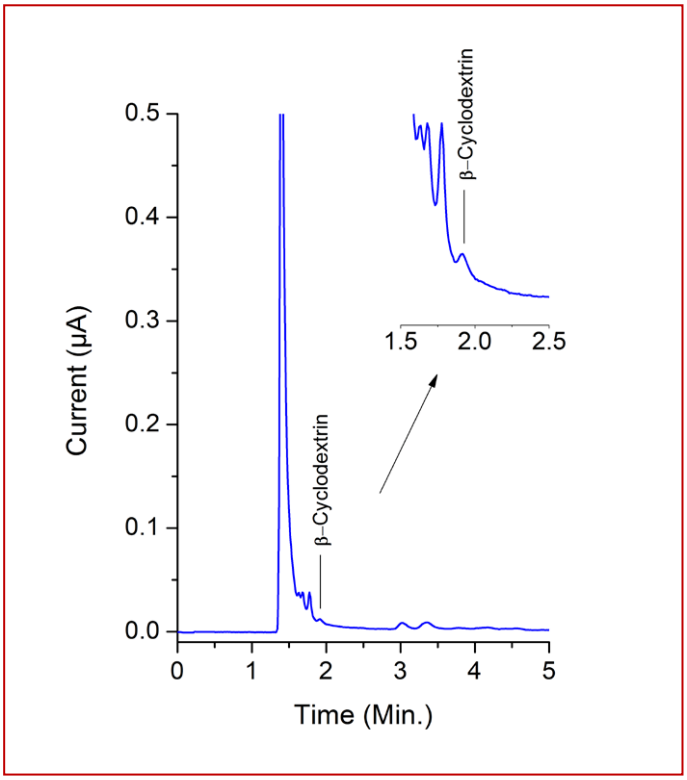


Figure 3: Chromatogram of a 20 µL injection of a 2 mg/mL solution of sample OS15979 in water. Inset (top-right): zoom-in on the β-Cyclodextrin response.

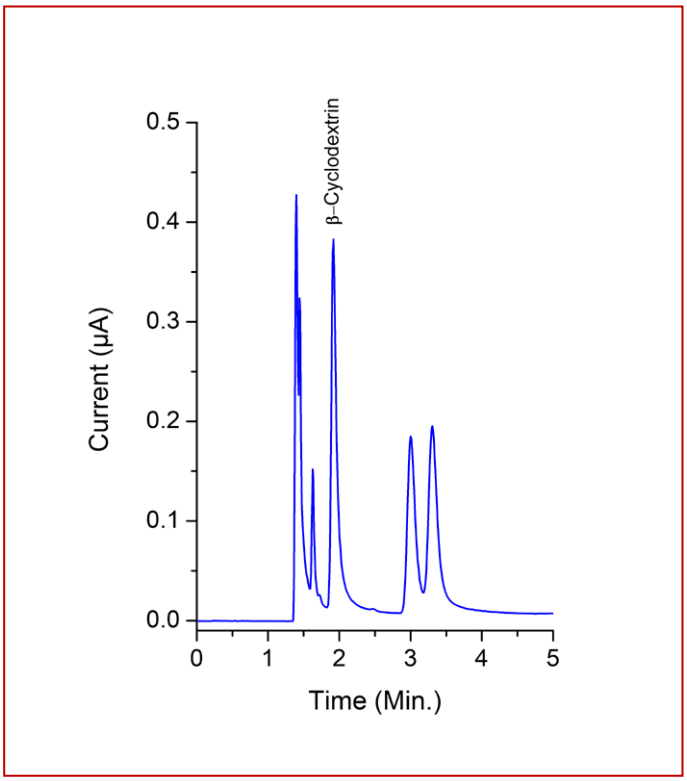
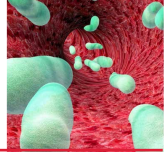


Figure 4: Chromatogram of a 20 µL injection of a 2 mg/mL solution of sample OS15979 in water. Inset (top-right): zoom-in on the β-Cyclodextrin response.



less β-Cyclodextrin impurity than sample 1065550.

The percentage of β-Cyclodextrin in the portion of β-Cyclodextrin sulfobutyl ether sodium was calculated as specified in the USP monograph:

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

Where:

r_U = β-Cyclodextrin peak area obtain from the chromatogram of the sample solution (mg/mL)

r_S = β-Cyclodextrin peak area obtain from the chromatogram of the standard solution (mg/mL)

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

C_U = Concentration of β-Cyclodextrin in the sample solution (mg/mL)

F = Conversion factor (10^{-3} mg/µg)

The results and the USP acceptance criteria for the β-Cyclodextrin contents in Betadex sulfobutyl ether sodium are given in Table 4. The content of β-Cyclodextrin in both analyzed Betadex sulfobutyl ether sodium samples was within the specified limit of the USP monograph.

Table 4

Limit of β-Cyclodextrin (USP)

Betadex sulfobutyl ether sample	*USP criterium*	*Measured*
1065550	<0.1%	0.049%
OS15979	<0.1%	<0.001%

References

1. Cyclodextrin, Wikipedia, https://en.wikipedia.org/wiki/Cyclodextrin
2. A. Vyas, S. Saraf, S.Saraf, Cyclodextrin based novel drug delivery systems, J. Incl. Phenom. Macrocycl. Chem., 62 (2008), 23–42
3. T.Challa, A. Ahuja, J. Ali, R.K. Khar, Cyclodextrins in Drug De - livery: An Updated Review, AAPS PharmSciTech, 6 (2005), E329-E357
4. 4M. E. Brewster, T. Loftsson, Cyclodextrins as pharmaceutical solubilizers, Advanced Drug Delivery Reviews, 59 (2007), 645–666
5. W.R. LaCourse, Pulsed Electrochemical Detection in High Per- formance Liquid Chromatography, John Wiley & Sons, New York, 1ed, 1997
6. J. Haginaka, Y. Nishimura, J. Wakai, H. Yasuda, K. Koizumi, T. Nomura, Determination of cyclodextrins and branched cy- clodextrins by reversed-phase chromatography with pulsed amperometric detection and a membrane reactor, Anal. Bio- chem. 179 (1989), 336-40.
7. T. Toropainen, P. Jarho, M. Lehtonen, P. Keski-Rahkonen, H. Raatikainen, T. Jarvinen, Quantitative analysis of natural cyclodextrins by high-performance liquid chromatography with pulsed amperometric detection: Application to cell per- meation study, Journal of Chromatography B, 867 (2008), 90–98
8. Betadex Sulfobutyl Ether Sodium, The United States Pharmacopeia 38th ed., National Formulary 33th, United States Pharmacopeial Convention, Rockville MD, (2015), 6546-6548

Conclusion

The ALEXYS HPAEC-PAD Analyzer offers a tailored solution for the impurity analysis of β-cyclodextrin in Betadex sulfobutyl ether sodium following the official method of the USP

Figure 5: Recommended instrument configuration for this application: the ALEXYS HPAEC-PAD Analyzer with Solvent Switch Valve.
The system consists of a P6.1L pump with integrated degasser and Solvent Switch Valve (SSV) for the option to run step gradients, an AS6.1L autosampler, an ET 210 Eluent tray for helium blanketing, and the DECADE Elite electrochemical detector. The ALEXYS HPAEC-PAD Analyzer can be operated under different Chromatography Data System (CDS) software: DataApex™ Clarity™ CDS (version 8.3 and up) or Thermo Scientific™ Chromeleon™ CDS (version 7.2 SR 5 and up).

Ordering information

Detector only	
176.0035A	DECADE Elite SCC electrochemical detector
102.4325EP	Flexcell Au HyREF with SS AUX
110.1300	sb REF (Ag/AgCl)
250.1045	Flattening/polishing kit for metal WE
Recommended ALEXYS analyzer + parts	
180.0055W	ALEXYS HPAEC-PAD Analyzer - with Solvent Switch Valve
102.4325EP	Flexcell Au HyREF with SS AUX
110.1300	sb REF (Ag/AgCl)
250.1045	Flattening/polishing kit for metal WE
Software	
195.0035[#]	Clarity CDS single instr. incl LC, AS module

#) optional: Antec ECD drivers for use with Chromeleon CDS , OpenLAB CDS or OpenLAB Chemstation CDS are available.

For research purpose only. The information shown in this communication is solely to demonstrate the applicability of the ALEXYS system and DECADE Elite detector. The actual performance may be affected by factors beyond Antec's control. Specifications mentioned in this application note are subject to change without further notice.

Antec Scientific (USA)
info@AntecScientific.com
www.AntecScientific.com
T 888 572 0012

Antec Scientific (worldwide)
info@AntecScientific.com
www.AntecScientific.com
T +31 71 5813333