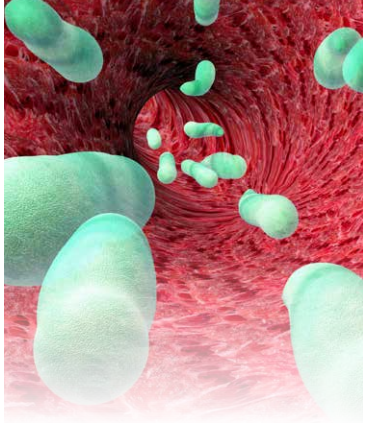
Application Note

ALEXYS - pharma



ALEXYS Analyzer for Highest Sensitivity in Neurotransmitter Analysis

Monoamines and Metabolites
- Noradrenaline
- Dopamine
- Serotonin
- 5-hydroxyindole acetic acid (5-HIAA)
- 3,4-dihydroxyphenylacetic acid (DOPAC)
- homovanillic acid (HVA)

OPA derivatized amines and amino acids
- GABA and Glutamate
- Histamine (LNAAs)
- 4-aminobutyrate (GABA)
- Glutamate (Glu)
- LNAAs

Choline and Acetylcholine
- Choline (Ch)
- Acetylcholine (ACh)

Markers for oxidative stress
- 3-nitro-L-Tyrosine
- 8-OH-DPAT

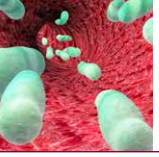
Glutathione and other thiols

Betadex Sulfobutyl Ether Sodium according to USP

- **Meets all requirements of U.S. Pharmacopeia 38-NF33, 2015**
- **ALEXYS HPAEC-PAD Analyzer**
- **Flow cell with Au working electrode and Ag Reference electrode**
- **Reproducible and robust**

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 which 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, Anion-Exchange Chromatography (HPAEC) in combination with Pulsed Amperometric Detection (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].



Betadex Sulfobutyl Ether Sodium according to USP

Summary

The contents of β-cyclodextrin in Sulfobutyl ether β-cyclodextrin 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.

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-exchange 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 %). The IonPac AS11, 250 x 4.0 mm ID analytical column which has a L61 packing was chosen for the method evaluation. The analytical column was used in combination with an IonPac AG11 guard column with the dimensions 50 x 4.0 mm.

Table 1

LC-EC Conditions	
HPLC	ALEXYS HPAEC-PAD Analyzer, High Pressure gradient
Column	IonPac AS11, 250 x 4 mm ID analytical column + IonPac AG11, 50 x 4 mm ID guard column (USP column packing L61)
Mobile phase A	25mM sodium hydroxide
Mobile phase B	250mM sodium hydroxide, 1M potassium nitrate.
Flow rate	1.0mL/min (gradient elution)
Vinjection	20 µL
Temperature	50°C for separation and detection
Flow cell	VT-03™ with Au WE, stainless steel AE and Ag RE, spacer 50 µm
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	ca. 0.2 µA
ADF	0.5 Hz
Range	2 µA

The analysis is based on a step-gradient profile as specified in the USP monograph, see 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.

The ALEXYS HPAEC-PAD analyzer was equipped with two pumps to enable high pressure binary gradient elution required for the column clean-up step. 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 sonicated and 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 to assure a reproducible analysis.

Table 2

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

Figure 1: ALEXYS HPAEC-PAD analyzer with binary gradient for β-cyclodextrins.

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). An Antec VT-03 electrochemical flow cell matching these requirements was used in this evaluation. A 3-step potential waveform is used as described in the USP monograph, see table 1. The cell current was typically about 0.2 µA with these PAD settings under the specified 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.

Sample preparation

Standard preparation: 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.

Sample preparation: Two commercial samples were obtained and analyzed:

(1) Sulfobutyl ether β-cyclodextrin sodium (Carbosynth, product code OS15979)
(2) USP Betadex Sulfobutyl Ether Sodium RS (USP, product code 1065550)

Both sample solutions were prepared in the following way: 10 mg of the 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.

System Suitability

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

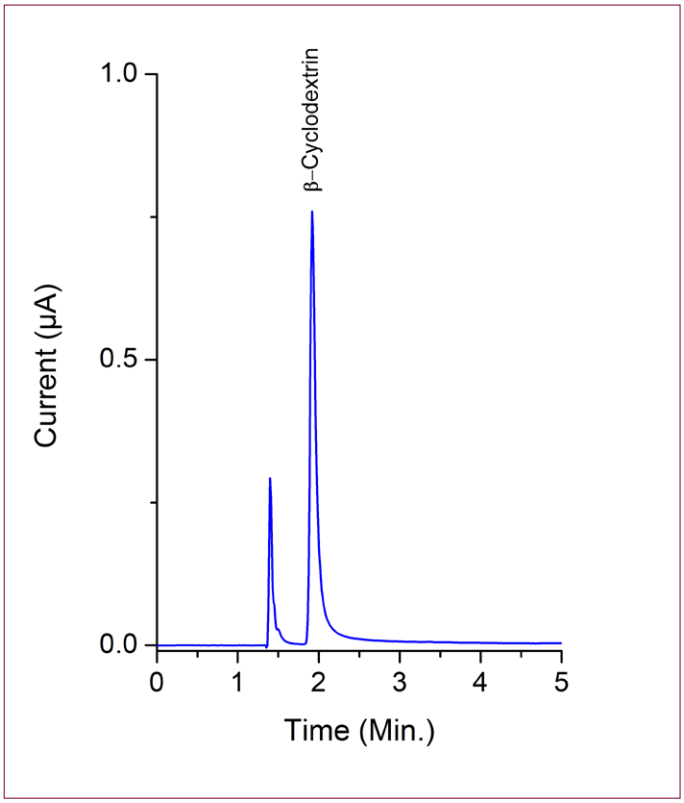


Figure 2: Chromatogram obtained with a 20 µL injection of a 2 µg/mL β-cyclodextrin RS standard in water.

In the USP monograph for Betadex sulfobutyl ether sodium the following system suitability requirement is specified: Relative standard deviation (RSD) not more than 5% (Area of β-cyclodextrin peak) for replicate injections of the standard solution.

Table 3

USP System Suitability Requirement		
Parameter	*USP criteria*	*Measured*
RSD peak area, n=6 (β-cyclodextrin)	<5%	0.3

The RSD requirement was evaluated by 6 repetitive injections of the USP standard solution, see table 3. It is evident that the system suitability requirement was met for the RSD of the peak area.

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) of the retention time, peak area and height were determined for 6 replicate injections of the USP β-cyclodextrin RS standard solution. The RSD's were <0.1%, 0.3% and 0.7%, respectively for the Streptomycin peak. 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

As an example two commercial β-Cyclodextrin sulfobutyl ether sodium salt samples were analyzed: (1) Carbosyth, product code OS15979 and (2) USP reference standard, product code 1065550. The samples are respectively abbreviated as sample OS15979 and 1065550 from this point onwards. The chromatograms from both sample solutions are shown in figure 3 and 4.

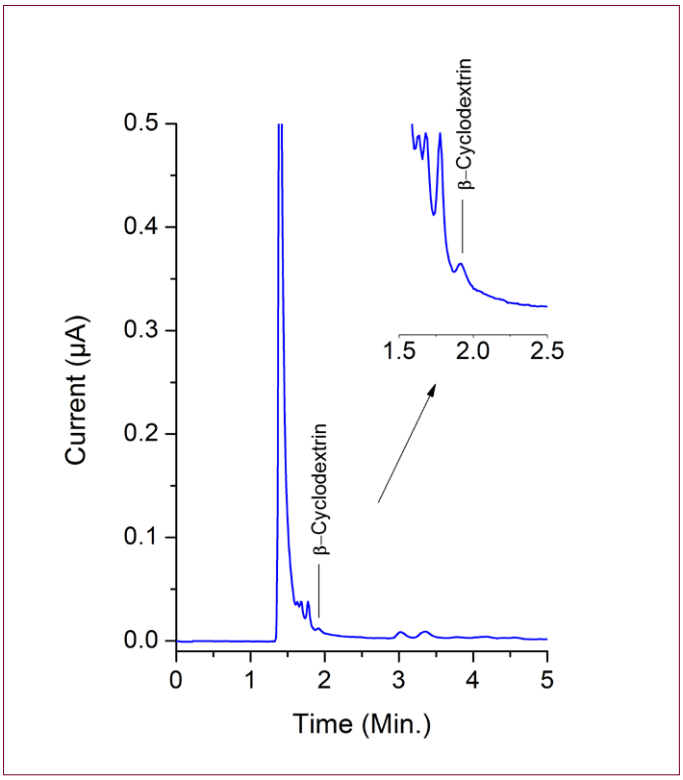


Figure 3: Chromatogram of a 20 µL injection of a 2 mg/mL solution of sample OS15979 in water. Inset (top-right): zoom-in on the β-Cyclodextrin response.

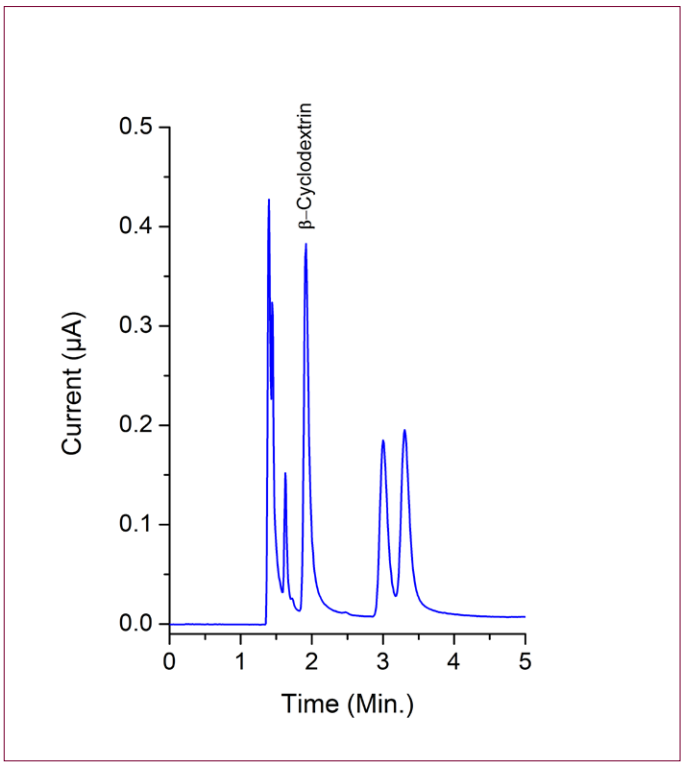


Figure 4: Chromatogram of a 20 µL injection of a 2 mg/mL solution of sample 1065550 in water.

It is evident from the response in both chromatograms that sample OS15979 contains significantly less β-Cyclodextrin impurity than sample 1065550. The percentage of β-Cyclodextrin in the portion of β-Cyclodextrin sulfobutyl ether sodium is calculated as specified in the USP monograph:

Percentage = (rU / rS) x (CS / CU) x F x 100

Where:

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

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

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

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

F = Conversion factor (10-3 mg/µg)

The USP acceptance criteria for the β-Cyclodextrin contents in Betadex sulfobutyl ether sodium is that the product should contain less than 0.1% β-Cyclodextrin. The result for both samples are listed in table 4.

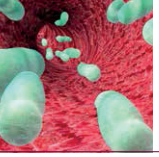
Table 4

Limit of β-Cyclodextrin USP		
Betadex sulfobutyl ether sample	*USP criteria %*	*Measured*
1065550	<0.1	0.049
OS15979	<0.1	<0.001

The contents of β-Cyclodextrin in both analyzed Betadex sulfobutyl ether sodium samples was within the specified limit of the USP monograph.

Conclusion

The ALEXYS HPAEC-PAD Analyzer with high-pressure gradient option offers a tailored solution for the impurity analysis of β-cyclodextrin in Betadex sulfobutyl ether sodium using the official method of the USP.



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8. *Betadex Sulfobutyl Ether Sodium*, The United States Pharmacopeia 38th ed., National Formulary 33th, United States Pharmacopeial Convention, Rockville MD, (2015), 6546-6548

Ordering number

180.0055E	ALEXYS HPAEC-PAD Analyzer, HP gradient
250.1086	IonPac AG11 guard column, 50 x 4.0 mm ID
250.1087	IonPac AS11 analytical column, 250 x 4.0 mm ID