Average Degree of Substitution of Betadex Sulfobutyl Ether Sodium Using the Agilent 7100 Capillary Electrophoresis System

Application Note

Pharmaceuticals

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Abstract

The degree of substitution for sulfobutyl ether β-cyclodextrin (SBE-β-CD) has historically been characterized by nuclear magnetic resonance spectroscopy, but this approach provides gross values for the degree of substitution. The capillary electrophoresis method described here resolves the mixture of positional and regional isomers based on the degree of sulfo butyl ether substitution. Indirect UV detection has been used, where the detection of cyclodextrin is accomplished by a decrease in background absorbance of the background electrolyte buffer benzoic acid. Ten peaks of SBE-β-CD were well resolved with an optimum pH of 8.0 using an Agilent 50 µm id, 56 cm length bare fused silica capillary. The system suitability requirements were passed as per United States Pharmacopeia (USP), and acceptence criteria for averge degree of substitution 6.2 to 6.9 were met.

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Introduction

Betadex sulfobutyl ether sodium salt (SBE-β-CD Na) is a highly water-soluble anionic cyclodextrin derivative. It can easily form noncovalent inclusion complexes with drug molecules; thus it performs well in:

- Enhancing drug stability, solubility, and safety,
- Reducing drug toxicity and hemolysis
- Covering up bad smells
- Controlling drug release rate

SBE-β-CD Na has been used in injection, oral, nasal, and eye medication. Modification by charged functional units can improve the binding affinity of cyclodextrins for oppositely charged guests, because it has a special affinity for drugs with nitrogen elements. As per United States Pharmacopeia (USP), SBE-β-CD Na is prepared by alkylation of betadex using 1,4-butane sultone under basic conditions. The average degree of substitution in betadex is not less than 6.2, and not more than 6.9.

The sulfobutylether derivatives are a mixture of positional and regional isomers containing from one to 10 sulfobutylether substituent groups per one cyclodextrin backbone molecule. As the capillary electrophoresis technique involves a charge-based separation, it successfully resolves the isomers based on the degree of substitution, and allows for relative quantitation of the respective bands.

Analitical Technique

Instrumentation

- Agilent 7100 Capillary Electrophoresis (G7100A)
- Agilent standard bare fused silica capillary, 50 µm id × 56 cm effective length (G160-61211)

Reagents

- Agilent Ultra-pure CE water (p/n 5062-8578)
- Agilent N sodium hydroxide (p/n 5062-8575)
- Agilent 1.0 N sodium hydroxide (p/n 5062-8576)
- Tris buffer: 1.21 g of Tris (hydrooxymethyl) amino methane was dissolved in 100 mL CE water to give a concentration of 100 mM
- Running electrolyte: 3.66 g of Benzoic acid is dissolved in 950 mL CE water, pH adjusted to 8.0 with 100 mM Tris (hydrooxymethyl) amino methane buffer and final volume made up to 1,000 mL to give a concentration of 30 mM benzoic acid

Note: Since benzoic acid is immiscible in water, it was subjected to magnetic stirring with heating followed by sonication. This was repeated several times until it completely dissolved.

Sample preparation

Dissolve 10 mg of SBE-β-CD Na in 10 mL CE water to give a concentration of 10 mg/mL. Filter this solution using a 0.45 µ syringe, then inject it onto the CE.

Standard preparation

Dissolve 10 mg of USP SBE-β-CD Na RS in 10 mL of CE water to give a concentration of 10 mg/mL. Filter this solution using a 0.45 µ syringe, then inject it onto the CE.

Operating conditions

Mode	CE
Capillary temperature	25 °C
Applied voltage	0 to 30 kV linear ramp over 10 minutes, then at 30 kV for a further 20 minutes
Injection mode	Pressure
Injection size	34 mbar for 10 seconds, Inlet: Injection vial Outlet: Running electrolyte
Detection wavelength (Inverse UV detection)	360 nm, BW: 20 nm, Ref: 205 nm, BW: 10 nm
Capillary rinsing	
Daily basis before each analysis	0.1 N sodium hydroxide for 30 minutes, Water for 120 minutes Run electrolyte for 60 minutes
Prior to each injection	N sodium hydroxide for 2 minutes Run electrolyte for 3 minutes
For a new capillary*	1 M sodium hydroxide for 60 minutes Water rinse for 120 minutes

* Used only for a new capillary

Results and Discussion

Figure 1 shows a representative electropherogram of 10 peaks of varying degrees of substitution.

The approximate relative migration time for SBE-β-CD Na peaks I to X for standard and sample are compared against the USP reference in Table 1.

For each of SBE-β-CD Na peaks I–X, limit range (% peak area) was compared for the reference standard and sample against the USP method in Table 2.

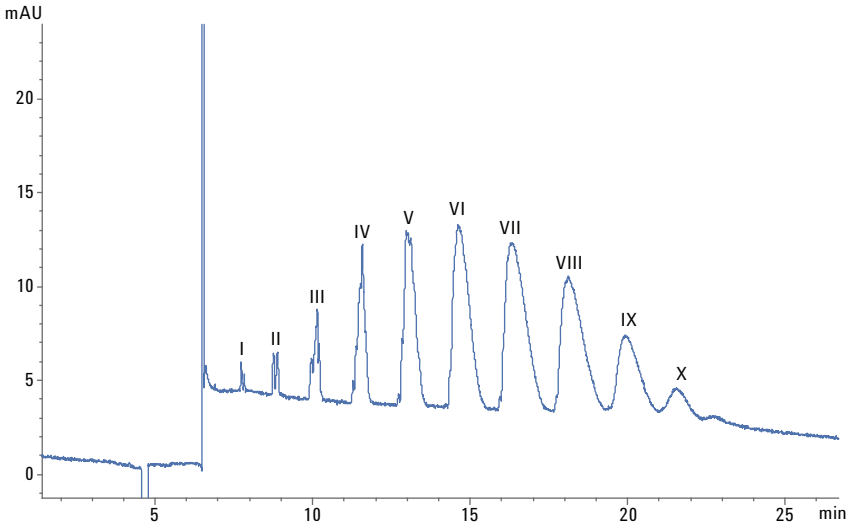

Figure 1. Representative electropherogram of sulfobutyl ether β-cyclodextrin (SBE-β-CD) using an Agilent 7100 CE.

Table 1. Approximate relative migration time for SBE-β-CD Na peaks I to X.

Betadex sulfobutyl ether sodium peaks I to X	Relative migration time (USP)	Relative migration time (Reference standard)	Relative migration time (Sample)
I	0.58	0.60	0.59
II	0.63	0.65	0.64
III	0.69	0.72	0.71
IV	0.77	0.79	0.78
V	0.83	0.85	0.85
VI	0.91	0.93	0.92
VII	1.00	1.00	1.00
VIII	1.10	1.09	1.10
IX	1.20	1.18	1.20
X	1.30	1.26	1.29

Table 2. Limit range values for SBE-β-CD Na peaks I–X.

Betadex sulfobutyl ether sodium peaks I to X	Limit range % peak area (USP)	Limit range % peak area (Reference standard)	Limit range % peak area (Sample)
I	0–0.3	0.03	0.02
II	0–0.9	0.23	0.16
III	0.5–5.0	1.65	1.73
IV	2.0–10.0	5.77	6.92
V	10.0–20.0	13.55	15.95
VI	15.0–25.0	23.00	23.96
VII	20.0–30.0	28.55	27.87
VIII	10.0–25.0	18.64	16.84
IX	2.0–10.0	6.85	5.60
X	0–4.0	1.67	0.94

1. Record the electropherograms and measure the peak responses for the betadex sulfobutyl ether sodium peaks (I to X).
2. Calculate the corrected peak area for each peak in electropherogram (Equation 1).
3. Normalize the recorded peak areas as a percentage of the total corrected substitution envelope area (Equation 2).
4. Determine the average degree of substitution using Equation 3.

Table 3 shows that the average degree of substitution for reference standard and sample are met within the USP acceptance criteria.

The resolution between the IX and X peaks was calculated as 1.51, which met the USP resolution requirement NLT 0.9 between IX and X peaks.

$$\textit{Corrected peak area (Ai)} = \frac{\textit{(peak area} \times \textit{effective capillary length (cm))}}{\textit{migration time}}$$

Equation 1.

$$\textit{Normalized peak area (NAi)} = \frac{Ai}{\Sigma Ai} \times 100$$

Equation 2.

$$\textit{Average degree of substitution} = \frac{\Sigma\textit{(level of substitution for peak} \times \textit{NAi)}}{100}$$

Equation 3.

Table 3. Average degree of substitution for reference standard and sample.

Entity	Averge degree of substitution
USP acceptence criteria	6.2–6.9
Reference standard	6.44
Sample	6.28

Conclusion

The determination of the pattern of composition for a mixture of mono to deca-substituted anionically charged cyclodextrin, the SBE-β-CDs, were successfully resolved on an Agilent 7100 Capillary Electrophoresis after optimizing parameters such as buffer pH, capillary temperature, and electric field strength.

Reference

1. Betadex Sulfobutyl Ether Sodium USP NF 33, Official Monograph, Betadex 6546-6550.

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