

Large-Scale Synthesis and Comprehensive Validation of 2-Hydroxypropyl- β -Cyclodextrin Equivalent to Commercial Product

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Received: 2 October 2025, Revised: 16 October 2025, Accepted: 1 November 2025, Published: 5 January 2026

Abstract

This study was a development from a previous study, which established the method for synthesizing 2-hydroxypropyl- β -cyclodextrin (HP β CD) from β -cyclodextrin and successfully applied it in large-scale production. The large-scale synthesis of HP β CD produced 1.38 ± 0.05 kg of 2-Hydroxypropyl- β -cyclodextrin, yielding $86.77 \pm 0.68\%$, and a degree of substitution of 5.91. The synthesis was monitored using thin-layer chromatography. The synthesized product was then structurally characterized using infrared spectroscopy, nuclear magnetic resonance spectroscopy, and mass spectroscopy, along with the commercial HP β CD. As a comparative analysis, the spectroscopic data revealed no significant structural differences between the synthesized HP β CD and a commercially available counterpart. These findings validate the developed synthesis method, highlighting its potential for industrial application and future research into the uses of HP β CD in drug development.

Keywords: β -cyclodextrin, Characterization, Hydroxypropyl- β -cyclodextrin, Structure, Synthesis

Introduction

In pharmaceutical research and development, a significant challenge lies in the poor solubility of active pharmaceutical ingredients (APIs), which have been estimated as nearly one-third of recently developed drug candidates exhibit inadequate solubility, and approximately half fail during pharmacokinetic trials [1]. Poorly soluble drugs often demonstrate slow absorption rates, insufficient bioavailability, and increased toxicity due to prolonged residence on mucosal surfaces. According to the Biopharmaceutics Classification System, for drugs with low aqueous solubility, when administered orally, the dissolution rate becomes the rate-limiting step controlling both the speed and extent of absorption [2]. Various techniques have been employed to improve the solubility and dissolution rate of poorly water-soluble drugs, including solid dispersions, nanocrystals, co-crystals, co-

evaporation, co-precipitation, polymer conjugation, surface adsorption, and complexation [3,4]. Among these methods, complexation with cyclodextrins has proven highly effective in improving both solubility and dissolution rates. Within the cyclodextrin family, beta-cyclodextrin (β CD) has been extensively studied for its ability to form complexes and enhance the solubility of APIs.

However, the relatively low aqueous solubility and potential renal toxicity of β CD limit its applicability [5,6]. Hydroxyalkyl derivatives of β CD, on the other hand, offer higher aqueous solubility and lower renal toxicity [7,8]. Importantly, their ability to form complexes with drug substances remains largely unchanged, leading to a significantly greater enhancement in drug solubility and dissolution rate compared to drug- β CD complexes [9]. Globally, hydroxyalkyl derivatives of β CD have been widely

adopted in drug research and development, with current research predominantly focusing on 2-hydroxypropyl- β -cyclodextrin (HP β CD) (**Figure 1**), with some emerging studies exploring 2-hydroxybutyl- β -cyclodextrin for improving API solubility [10,11]. Nevertheless, these materials have not yet been synthesized domestically in Vietnam and are not readily available in the local market. The successful domestic

production of and related derivatives will offer local researchers and chemical manufacturers greater autonomy, enabling them to bypass complex and costly technology transfer procedures, avoid high-priced commercial imports, and mitigate the supply chain availability constraints imposed by foreign suppliers, thus a step toward national self-sufficiency in key pharmaceutical excipients.

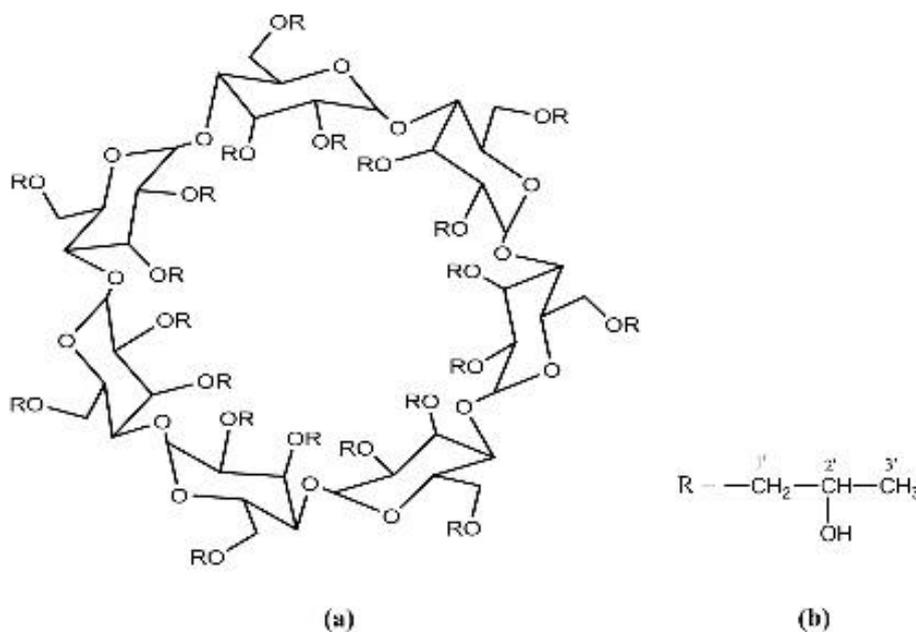


Figure 1 (a) Hydroxypropyl- β -cyclodextrin structure; (b): 1,2-propylene oxide as the substituent unit.

This study focused on providing a method for synthesizing HB β CD, detailing its structure, and comparing the synthesized HB β CD with a commercial one available in the Vietnamese market, thereby evaluating its potential for further drug development.

Materials and methods

Preparation of the large-scale synthesized 2-Hydroxypropyl- β -cyclodextrin (in kg)

The synthesis procedure was optimized and standardized for large-scale production; the structure of the large-scale product was also characterized in the previous research of Phung *et al.* [12]. The large-scale synthesis commenced by dissolving 1,135 g of β CD in 150 mL NaOH 1.5% (w/v) solution within a 2,000 mL-Quickfit® round-bottom glass (FR2L/3S) and refluxing for 1.5 h. Then, 565.5 mL of 1,2-propylene oxide (2.35 g, 99% purity, 40 mmol) was added portion-wise over a

period of 1 h, along with continuous refluxing at room temperature until the reaction reached completion.

The progress of the reaction was monitored by thin-layer chromatography (TLC) [13,14]. The silica gel GF254 plates were used as the stationary phase and composed of isopropanol, ethyl acetate, and water in a 5:2:2 (v/v/v) ratio as the mobile phase. Two separate TLC plates were prepared for β CD (10 μ L of 0.5% aqueous solution) and the HP β CD plate, on which 10 μ L of the reaction mixture and 10 μ L of a 0.5% aqueous solution of an HP β CD reference standard were spotted. The spots on both plates were visualized using saturated iodine vapor. The reaction was considered complete when, on the chromatogram of the first plate, the β CD spot was no longer detected in the reaction mixture. On the second plate, the criteria for completion were that the spot corresponding to the formed product did not exhibit

tailing and showed a similar R_f value to that of the HP β CD reference standard.

After neutralization at pH 7.0 by 1 M HCl, the reaction mixture was concentrated *in vacuo* (80 °C). The residue was dissolved in 99.5% ethanol, filtered to remove NaCl, and the product precipitated from the filtrate using acetone (0 - 5 °C). The precipitate was purified by multiple reprecipitations from 99.5% ethanol/acetone to yield a fine powder, subsequently dried *in vacuo* (80 °C) to constant weight. This synthesized HP β CD was then ready for further analysis.

Characterization of synthesized and commercial 2-hydroxypropyl- β -cyclodextrin

The product obtained from the initial reaction stage, after purification to ensure adequate purity, was subjected to spectroscopic analysis, including Infrared (IR) spectroscopy, ^1H and ^{13}C Nuclear Magnetic Resonance (NMR) spectroscopy, and Mass Spectrometry (MS) to confirm its structure. The standard HP β CD used as a reference for this comparison was purchased from Roquette (France), which was aligned with the European. Ph/USP standard.

Infrared spectroscopy

Infrared Spectroscopy (IR) spectra were recorded using the potassium bromide pellet technique [15]. For sample preparation, approximately 1 - 2 mg of the test substance was intimately ground with 100 - 200 mg of previously dried, spectroscopic grade KBr powder. This mixture was then uniformly spread in a suitable die and compressed under vacuum at a pressure of approximately 800 MPa to form a 13 mm diameter pellet, designed to yield a spectrum of appropriate intensity. The IR spectrum of the sample was acquired, and the presence of characteristic absorption bands for O-H, C-H, and C-O functional groups was examined. The obtained spectrum was subsequently compared with the IR spectrum of an HP β CD reference standard.

Nuclear magnetic resonance spectroscopy

^1H -NMR, ^{13}C -NMR, Distortionless Enhancement by Polarization Transfer (DEPT), Correlation Spectroscopy (COSY), Heteronuclear Single Quantum Coherence (HSQC), and Heteronuclear Multiple Bond Correlation (HMBC) spectra were acquired in deuterium oxide D_2O as the solvent [16]. Measurements were performed on a Bruker AC 500 MHz NMR spectrometer. These analyses were conducted to determine H-H and H-C correlations and to elucidate the structure of the synthesized product. The resulting spectra were compared with those of β -cyclodextrin (β CD) and commercially available HP β CD.

Mass spectrometry

Mass spectra (MS) were obtained using an electrospray ionization (ESI) technique [17]. Samples were dissolved in methanol and analyzed over a mass-to-charge (m/z) range of 0 to 2,000. This analysis aimed to determine the distribution of HP β CD molecules with varying degrees of substitution (DS) within the synthesized product.

Results and discussion

Large-scale production of 2-Hydroxypropyl- β -cyclodextrin

TLC analysis yielded distinct results (**Figure 2**) for the starting material, the synthesized product, and the commercial product. The chromatogram of the β CD starting material displayed a single, discrete circular spot with a retention factor (R_f) value of 0.27. In contrast, the synthesized HP β CD exhibited a well-defined spot at $R_f = 0.72$, with no detectable trace of the unreacted β CD at $R_f = 0.27$. A comparative TLC analysis of the synthesized HP β CD standard revealed equivalent R_f values and comparable spot shapes, confirming the purity and identity of the synthesized product.

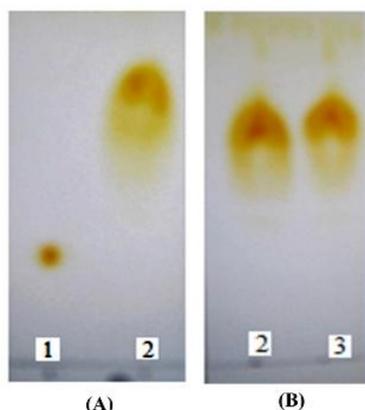


Figure 2 Chromatograms of (A): synthesized HP β CD vs. β CD and (B) synthesized HP β CD vs. commercial HP β CD, where (1): β CD, (2): synthesized HP β CD and (3) commercial HP β CD.

The large-scale synthesis of HP β CD was triplicated, given the results recorded in **Table 1**. The average quantity was calculated to be 1.38 ± 0.05 kg of the desired product, yielding $86.77 \pm 0.68\%$, and a DS

of 5.91, which was approximately equal to the record previous study of Truyen *et al.* [12] with 1.28 kg product, yielding 86.79%, and a DS of 5.91 [12].

Table 1 Large-scale production of HP β CD in triplicate under the same conditions.

	HP β CD (kg)	Yield (%)	Degree of substitution
Replicate 1	1.42	87.46	5.96
Replicate 2	1.39	86.75	5.87
Replicate 3	1.32	86.11	5.9
Mean \pm STEM	1.38 ± 0.05	86.77 ± 0.68	5.91 ± 0.05

Comparison of the synthesized and commercial HP β CD

Infrared spectroscopy

The IR absorbance peaks showed absolute similarities in all analyzed functional groups between the synthesized and commercial HP β CD, functional groups at their respective characteristic wave numbers are presented in **Figure 3** and described in **Table 2**. The

spectrum of the synthesized HP β CD exhibited characteristic absorption maxima (or peaks/bands) exactly corresponding to O-H at $3,384.8 \text{ cm}^{-1}$, C-H at $2,927.7 \text{ cm}^{-1}$, and the C-O at $1,031.8 \text{ cm}^{-1}$ for both samples. Moreover, the signals of the C-O-C bond were at $1,159.1$ and $1,158.2 \text{ cm}^{-1}$, interpreting this functional group in the synthesized and commercial HP β CD, respectively.

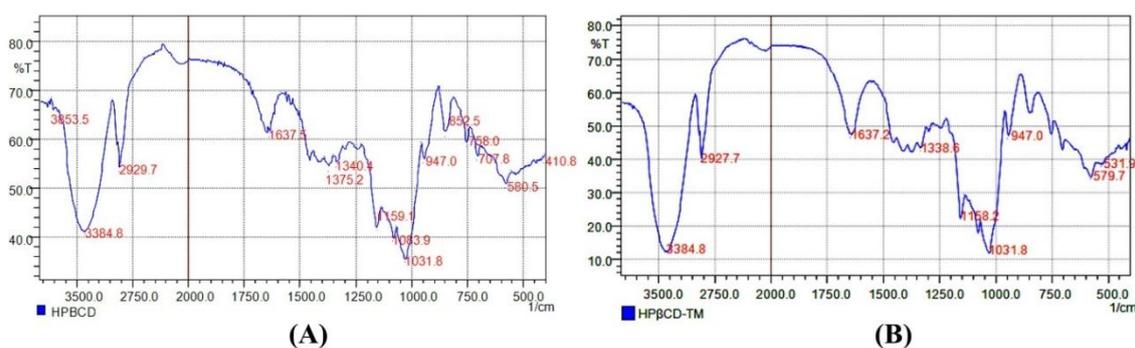


Figure 3 Infrared spectrum of: (A)- synthesized and (B): commercial HP β CD.

Table 2 IR spectra of the synthesized and commercially available HP β CD.

Functional groups	Absorbance peaks (cm ⁻¹)	
	Synthesized HP β CD	Commercial HP β CD
O-H	3,384.8	3,384.8
C-H	2,929.7	2,927.7
C-O	1,031.8	1,031.8

Nuclear magnetic resonance spectroscopy

A comparison of the 2 samples presented in **Figure 4** and **Table 3** revealed minor variations in their ¹H and ¹³C chemical shifts (for ¹H and ¹³C, respectively). These differences are likely due to variations in the DS between the synthesized and commercial products. Nevertheless, the overall comparison of ¹H and ¹³C-NMR chemical shift data supports the conclusion that the synthesized HP β CD has a structure consistent with that anticipated.

The NMR data of ¹H and ¹³C for the synthesized HP β CD (A) and (B) closely matched those of the commercial reference (C) and (D), affirming the successful synthesis, by clarifying as both ¹H spectra (**Figures 4(A)** and **4(C)**) exhibited the distinctive anomeric proton H-1 signal as a doublet (labeled '1')

consistently positioned near δ 5.00 ppm, confirming the characteristic α -(1 \rightarrow 4) glycosidic linkage of the cyclodextrin core.

Furthermore, the ¹³C spectra (**Figures 4(B)** and **4(D)**) displayed high structural similarity, particularly in the pyranose ring carbons (C₁ through C₆) clustered between δ 60 and δ 102 ppm (**Table 3**). Critically, the signals for the hydroxypropyl side chain - the methyl (3') and methylene (2' and 1') carbons - were present at identical chemical shifts ($\delta \sim 18$ and $\delta \sim 60 - 70$ ppm), demonstrating that the required substitution occurred with the same structural outcome as the commercial product. This overall spectral congruence provided strong evidence for the structural identity of the synthesized material.

Table 3 Chemical shift data from ¹H and ¹³C-NMR spectroscopy for synthesized and commercial HP β CD.

Position	Synthesized HP β CD		Commercial HP β CD	
	¹³ C-NMR	¹ H-NMR	¹³ C-NMR	¹ H-NMR
1	101.76	5.16	101.81	5.00
2	72.13	3.72	72.08	3.56
3	73.15	4.03	73.10	3.88
4	80.96	3.67	81.10	3.52
5	71.88	3.94	71.83	3.74
6	60.41	3.96	60.32	3.79
1' _{1,2} (a)	76.93	3.81	76.93	3.76
1' _{1,2} (b)	76.27	3.61	76.22	3.46
2' ₁	66.78	4.10	66.70	3.40
2' ₂	66.34	4.09	66.40	3.90
3' ₁	18.42	1.11, singlet	18.33	1.07, singlet
3' ₂	18.08		18.07	

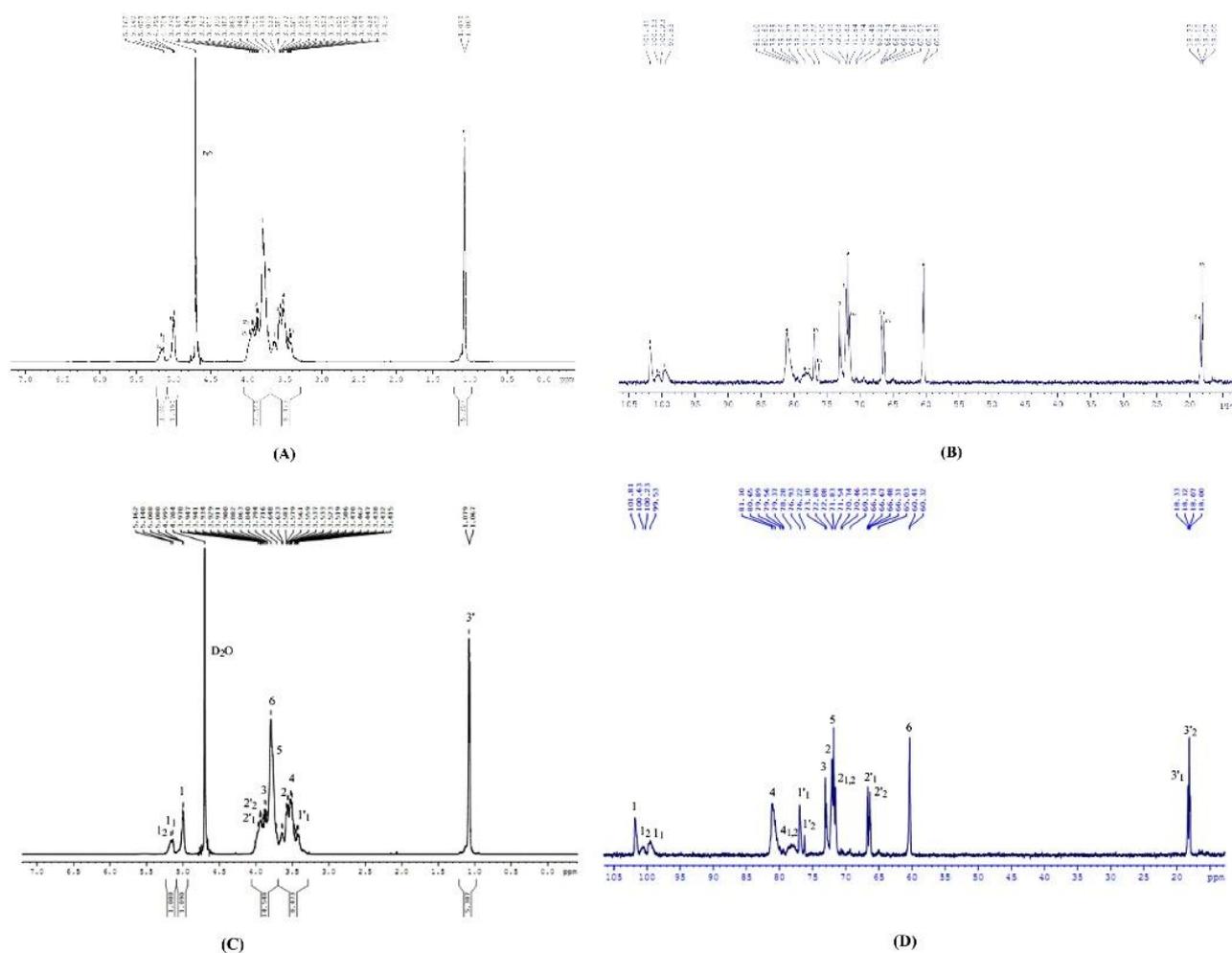


Figure 4 Nuclear magnetic resonance spectrum in D₂O of synthesized HPβCD as (A)- ¹H and (B)- ¹³C signals; and commercial HPβCD as (C)- ¹H and (D)- ¹³C signals.

Based on the initial information obtained from βCD described in Phung *et al.* [13], the analysis of the synthesized HPβCD spectrum also commenced from its ¹³C NMR data. The general principle governing this substitution involved the C-1 atom at the reactive end of the 1,2-propylene oxide molecule interacting with either the OH-C-2 or OH-C-3 hydroxyl groups of βCD to form -C(2)-O-R or -C(3)-O-R linkages (where R was -CH₂-CHOH-CH₃) under low alkaline concentrations. Obviously, at high alkaline concentrations, the substitution preferentially occurred at the OH-C-6 group, forming a -C(6)-O-R linkage.

However, as investigated by Sun *et al.* [18], substitution at OH-C-6 (a primary alcohol) was considerably challenging and necessitated specific reaction conditions. Consequently, in the substitution

process conducted under low alkaline conditions herein, the obtained product exclusively featured substitution at the OH groups at positions C-2 and C-3 of each of the seven entirely homogeneous glucopyranose units of βCD. As the substitution process occurred randomly across the glucose units, it was possible for some units to be singly substituted, others doubly substituted, and some to remain unsubstituted, unless all seven glucose units were doubly substituted (at 100% efficiency) [19].

Thus, the ¹H or ¹³C NMR signals of HPβCD, such as ¹³C-1 or ¹H-1, would split into 3 distinct peaks: one peak corresponding to the unsubstituted glucose unit (labelled as '1', representing no substitution), one peak (labelled '1₁') for the singly substituted unit, and one peak (labelled '1₂') for the doubly substituted unit, principally, this 3-fold splitting occurs for every ¹H or

^{13}C signal. However, spectral broadening can lead to signal overlap due to peak expansion, particularly in ^1H -

NMR. For instance, the H-5 and H-6 signals of unsubstituted βCD already exhibit overlap.

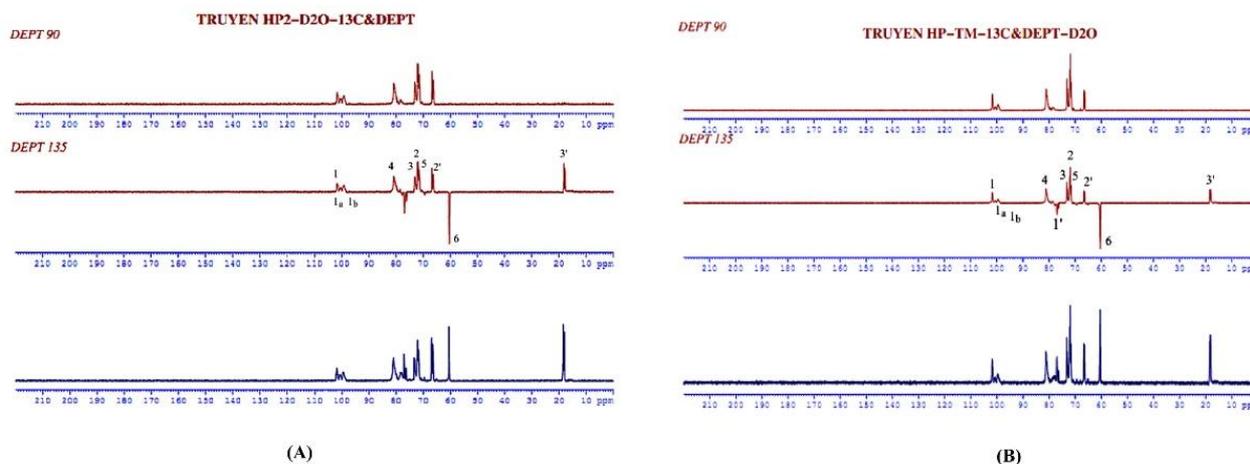


Figure 5 ^{13}C -NMR spectrum and DEPT of (A) synthesized and (B) commercial HP β CD in D_2O .

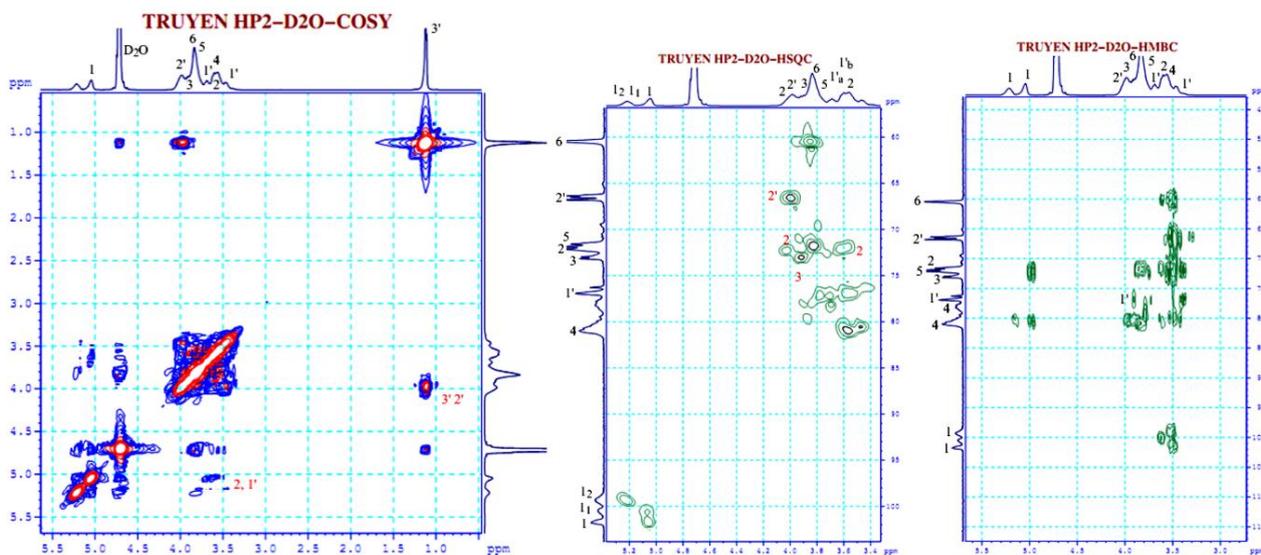


Figure 6 COSY, HMBC, and HSQC NMR spectra of the synthesized HP β CD measured in D_2O .

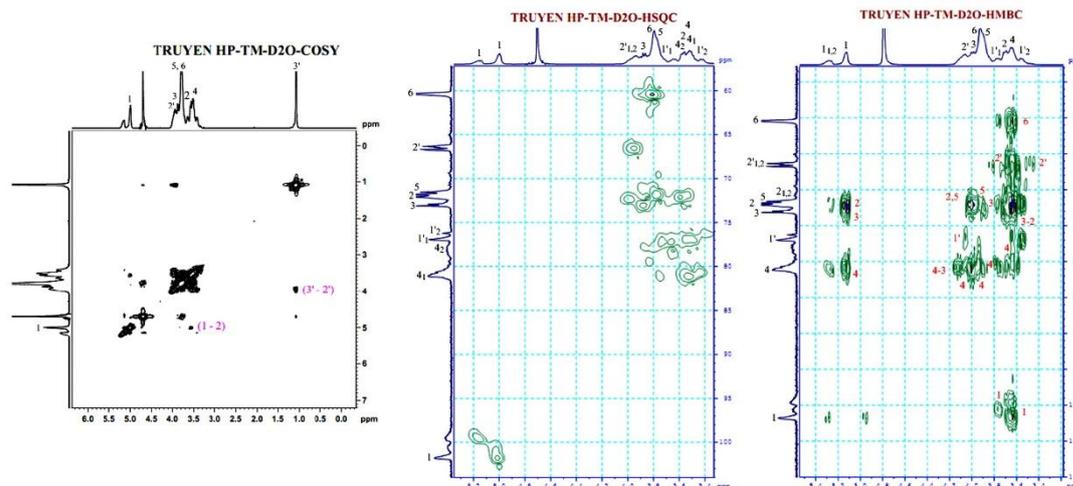


Figure 7 COSY, HMBC, and HSQC-NMR spectra of the commercial HP β CD measured in D₂O.

Table 4 NMR spectrum of commercial HP β CD in D₂O, including ¹H and ¹³C. ¹³C spectrum DEPT (90, 135), at a frequency of 125 MHz. ¹H, COSY, HSQC, HMBC spectra at a frequency of 500 MHz. (T = 297 K).

Position	Functional group	δ_C ppm	δ_H ppm	$J_{(Hz)}$ – signals (ppm)	COSY H→H	HMBC H→C
1	–O–CH–O–	101.81		5.00 *		
1 ₁	–O–CH–O–	99.53		5,140 ** (1 ₂)	2	2,3,4
1 ₂	–O–CH–O–	100.63		5,162 ** (1 ₁)		
2	–CH–O–	72.08		3.560 *		
2 _{1,2}	–CH–O–	71.54		3.580 *		2',3,4,1
3	–CH–O–	73.10		3.882 *		
3 _{1,2}	–CH–O–	72.89		3.863 *		2,5,4
4	–O–CH–	81.10		3.520 *	Overlapped	
4 _{1,2}	–O–CH–	79.56		3.535 *		1,2,3,5,6
5,5 _{1,2}	–CH–O–	71.83		3.74 ** (6 and 1' ₂)		3,4
6,6 _{1,2}	–CH ₂ –O–	60.32		3.794 ** (5 and 3)		5,4
1' _{1,2} (a*)	–O–CH ₂ –	76.93		3.755 ** (2 and 5)		
1' _{1,2} (b*)	–O–CH ₂ –	76.22		3.455 ** (4)		2',2,1
2' ₁	–CH–O–	66.70		3.95 ** (2' ₂)	3'	
2' ₂	–CH–O–	66.40		3.90 ** (2' ₁)	3'	1'
3' ₁	–CH ₃	18.33			2'	
3' ₂	–CH ₃	18.07		1.073 * (3' ₁ and 3' ₂)	2'	2,1

* Broadened singlet signals

** Overlapped signals

Table 5 NMR spectrum of synthesized HPβCD in D₂O, including ¹H and ¹³C. ¹³C spectrum DEPT (90, 135), at a frequency of 125 MHz. ¹H, COSY, HSQC, HMBC spectra at a frequency of 500 MHz. (T = 302 K).

Position	Functional group	δ_C ppm	δ_H ppm	$J_{(Hz)}$ – signals (ppm)	COSY H→H	HMBC H→C
1	–O–CH–O–	101.76		5.155 *		
1 ₁	–O–CH–O–	99.32		5.296 *	2,1	2,3,4
1 ₂	–O–CH–O–	100.53		5.317 *		
2	–CH–O–	72.13		3.723 *		
2 _{1,2}	–CH–O–	71.58		3.723 *		
3	–CH–O–	73.15		4.033 *	Overlapped	2,5,4
3 _{1,3}	–CH–O–	72.96		4.014 *		
4	–O–CH–	80.96		3.665 *		1,2,3,5,6
4 _{1,4}	–O–CH–	79.89		3.682 *		
5,5 _{1,5}	–CH–O–	71.88		3.935 ** (6)		
6,6 _{1,6}	–CH ₂ –O–	60.41		3.957 ** 5 and 3		
1' _{1,2} (a*)	–O–CH ₂ –	76.93		3.810 ** (2 and 5)	1,2'	2',2,1
1' _{1,2} (b*)	–O–CH ₂ –	76.27		3.607 ** (4)	1,2'	
2' ₁	–CH–O–	66.78		4.100 ** (2' ₂)	3',1'	1'
2' ₂	–CH–O–	66.34		4.087 ** (2' ₁)	3',1'	
3' ₁	–CH ₃	18.42		1.113 *	2'	2,1
3' ₂	–CH ₃	18.08			2'	

* Broadened singlet signals
** Overlapped signals

In the data presented in **Tables 4** and **5**, the primary analytical challenge was the ¹H NMR spectrum, where signals, apart from H-1 of βCD and CH₃-(3') of HPβCD, displayed significant broadening and overlap. This was exacerbated by the 2 non-equivalent protons of the –CH₂-(1') group, distinctly resolved in the HSQC spectrum. The clear separation of H-2/H-4 and H-5/H-6 clusters in the βCD spectrum, HPβCD's H-1'(a) and H-1'(b) signals intruded, obscuring most proton signals except H-1 and H-3(3'). The NMR signals of both the synthesized and commercial HPβCD were assigned based on correlation spectra (¹H, ¹³C, COSY, HSQC, and HMBC), and the atom notation in the structural formula (C₁–C₆ and H–H₆ for the glucopyranose ring, and C1'–C3'/H1'–H3' for the 2-hydroxypropyl substituent) is consistent with the spectra data.

In the ¹H-NMR spectra, the characteristic anomeric proton (H1) of β-cyclodextrin appeared at δ 4.83 - 5.07 ppm, confirming the retention of the glucopyranose framework. The multiplet signals at δ 3.2

- 4.0 ppm corresponded to H2–H6 of the glucose units and overlapped with the protons of the 2-hydroxypropyl groups (H1', H2', H3'). In the ¹³C-NMR spectra, the anomeric carbon (C-1) appeared around δ 102.5 - 104.0 ppm for both samples, while signals of the ring carbons C-2 to C-6 were observed in the range δ 60 - 82 ppm. The additional peaks at δ 72.3, 74.9, and 64.5 ppm were assigned to the carbons of the 2-hydroxypropyl substituents (C1', C2' and C3'), confirming successful etherification in the secondary hydroxyl groups (mainly C2 and C3 positions).

Despite these ¹H NMR complexities, reliable data table construction was achieved through ¹³C NMR (including DEPT 90 and DEPT 135) (**Figure 5**) and 2D-HSQC correlation spectroscopy (**Figures 6** and **7**). This robust dataset formed the basis for confirming the accurate synthesis of HPβCD. A comprehensive comparative analysis of synthesized and commercial HPβCD confirmed their structural identity. Minor,

acceptable variations in $\delta^1\text{H}$ and ^{13}C values were attributed to their differing DS.

Overall, the commercial HP β CD spectrum exhibited slightly better resolution than that of the synthesized HP β CD. This marginal difference was likely due to the measurement temperatures: the commercial sample was analyzed at 297 K, while the synthesized sample was measured at 302 K, meaning higher temperatures increase molecular motion, which can negatively impact spectral resolution. A 5 K difference in temperature is sufficient to account for the observed disparity in resolution, as mentioned in the previous study of Trainor *et al.* [20].

In ^1H -NMR spectroscopy, the intensity of a signal was directly proportional to the concentration (and thus the number) of protons contributing to that signal. For instance, within a single functional group such as $-\text{CH}_2-\text{CHOH}-\text{CH}_3$, if the signal intensity of the $-\text{CH}_2-$ group was assigned a relative value of 2 units, then the signal intensity of the $-\text{CH}_3$ group had to inherently be 3 units, reflecting the ratio of protons. In case a single glucopyranose unit was substituted at, for example, OH-C-2 by one 2-hydroxypropyl group, the intensity of the substituent's $-\text{CH}_3$ signal would be 3 times that of the anomeric H-1 signal from a single glucopyranose unit in β CD. Consequently, for complete substitution of one glucopyranose unit, the ratio of (1/3 the intensity of the 2-hydroxypropyl's $-\text{CH}_3$ -(3') signal) to the intensity of one glucopyranose unit's H-1 signal should be unity.

Based on this, the Molar Substitution (MS) can be calculated using the formula:

$$\text{MS} = \frac{A_1}{3 \times A_2}$$

where, A_1 is the intensity of the $-\text{CH}_3$ -(3') signal; A_2 is the intensity of the H-1 signal; The DS is defined as $\text{DS} = 7 \times \text{MS}$.

This convention was based on a product with exclusive single substitution (with 7 glucopyranose on each P β CD). However, our synthesized product exhibits substitution at both C-2 and C-3 positions, as detailed in **Table 5**. In the NMR spectra of HP β CD, particularly the ^{13}C spectrum, the C-1 and H-1 signals both split into 3 distinct peaks. This tripartite splitting signified the coexistence of unsubstituted, singly, and doubly

substituted β CD units; correspondingly, the ^1H and ^{13}C signals of the 2-hydroxypropyl substituent showed doublet splitting, indicating both 2-O-(2-hydroxypropyl) and 3-O-(2-hydroxypropyl) linkages. However, the $-\text{CH}_3$ -3' signal of the 2-hydroxypropyl group occasionally lacked this doublet splitting due to its remote position (4 σ bonds) from the substitution site. For calculating the MS and DS, the total intensity of the H-1 signals (unsubstituted, H-11 for single substitution, and H-12 for double substitution) was recruited. Similarly, for the $-\text{CH}_3$ -3' signal, the sum of intensities for $-\text{CH}_3$ -3'1 and $-\text{CH}_3$ -3'2 was utilized. In the synthesized HP β CD, while ^{13}C signals show clear splitting, the $\delta^1\text{H}$ signals for 3'-1 and 3'-2 overlap, precluding the use of ^{13}C signals for quantitative analysis due to their non-proportional intensity to concentration.

$$\text{MS} = \frac{5.163}{3 \times (1.000 + 1.229)} = 0.772$$

$$\text{DS} = 7 \times \text{MS} = 7 \times 0.772 = 5.404$$

Substitution efficiency (expressed as a percentage) [13] is a commonly employed metric in chemistry, taking the H-1 signal as an example, it splits into 3 distinct peaks based on its substitution. H-1 corresponds to the unsubstituted portion, H-1₁ to the singly substituted portion, and H-1₂ to the doubly substituted portion. Thus, when $I(1)$ represented the intensity of the unsubstituted H-1 signal, $I(1_1)$ would represent the intensity of the H-1₁ signal, and $I(1_2)$ would represent the intensity of the H-1₂ signal, the overall substitution efficiency (P) can be expressed as:

$$P(\%) = \frac{I(1_1) + I(1_2)}{I(1) + I(1_1) + I(1_2)} \times 100$$

Ideally, when peak separation was complete, individual efficiencies for the first substitution (P_1) and the second substitution (P_2) could be calculated. However, in ^1H -NMR spectra, the distinct resolution of H-1₁ and H-1₂ signals was typically absent. Thus, the total substitution efficiency (P) was calculated to be 44.86%, which was very high and described an effective design of the reaction. As shown in **Table 6**, the

synthesized HP β CD consistently displayed superior indices across all evaluated parameters.

$$P = \frac{1.000}{1.000 + 1.229} \times 100 = 44.86 \%$$

Table 6 Comparison of engineered parameters of synthesized and commercial HP β CD.

Parameters	Synthesized HP β CD	Commercial HP β CD
Molar Substitution (MS)	0.772	$MS = \frac{5.307}{3 \times (1.000 + 1.890)} = 0.612$
Degree of Substitution (DS)	5.404	$DS = 7 \times MS = 7 \times 0.612 = 4.285$
Efficiency (P)	44.860%	$P = \frac{1.000}{1.000 + 1.890} \times 100 = 34.60\%$

Mass spectrophotometry

The synthesized HP β CD exhibited isomers with molecular masses corresponding to 9 different degrees of substitution, continuously distributed from 1 to 9 through the calculation and listed in **Table 7**. The molecular formula for β CD is C₆H₁₀O₅ with a molecular weight of 1,134.994 g/mol. The β CD molecule has the substituent group -CH₂-CHOH-CH₃, having a molecular weight of 59.08 g/mol. During the initial substitution, the C-2 hydroxyl group of β CD reacts with C-1' of 1,2-propylene oxide, forming a -C-O-CH₂-linkage. This reaction involves the loss of one hydrogen atom from the β CD molecule and the gain of one hydrogen atom by the 1,2-propylene oxide molecule. Thus, for the first substitution, the mass of the β CD portion in singly-substituted HP β CD is 1,133.994 Da (losing one H atom), and conversely, the mass of the 1,2-propylene oxide portion in singly-substituted HP β CD is 58.08 + 1 = 59.08 Da (adding one H atom). Consequently, the molecular mass of the molecule resulting to be: $M_{\text{HP}\beta\text{CD}}(1) = 1,133.994 + 59.08 = 1,193.074$ Da. Then, in the second substitution reaction, the HP β CD framework (1) lost an additional hydrogen atom from a hydroxyl group at each substitution site, and the doubly substituted HP β CD became: $M_{\text{HP}\beta\text{CD}}(2) = M_{\text{HP}\beta\text{CD}}(1) - 1 + 59.08 = M_{\text{HP}\beta\text{CD}}(1) + 58.08$ Therefore, for a molecule of HP β CD that had undergone substitution reactions ($n \geq 1$), its molecular mass would be calculated in carbon units by the following Eq. (1):

$$M_{\text{HP}\beta\text{CD}}(n) = M_{\text{HP}\beta\text{CD}}(1) + (n - 1) \times 58.08 \quad (1)$$

where, n is the degrees of substitution.

In mass spectroscopy, spectral peaks are always recorded in ionic form. In the ES⁺ technique, common ionizing agents include H⁺, Na⁺, and K⁺. The specific fragment observed depends on the characteristics of the carrier gas and the fragmentation procedure employed during spectrum acquisition. In the ES⁺ method, the Na⁺ ion is most commonly observed. These ions are generated by breaking a bond within the molecule under investigation, transforming the neutral molecule into an ion. In the context of HP β CD analysis by mass spectroscopy, the molecule with the highest degree of substitution (and thus the largest mass) will typically be ionized. This ionized molecule then undergoes fragmentation, where one of its bonds is cleaved. This scission results in 2 fragments: one fragment may combine with a proton (H⁺), while the other will combine with a sodium ion (Na⁺) to form a characteristic molecular ion. Thus, a neutral molecule with a molecular mass of M, after fragmentation and ionization, will form an ion with a mass corresponding to the fragment plus any adducted species, typically represented as:

$$M^{+}_{m/z} = M + m_{\text{Na}} = M + 23 \quad (M^{+} = M \text{ v} \text{ } z = 1)$$

$$M^{+}_{m/z} = M + m_{\text{H}} + m_{\text{Na}} = M + 1 + 23$$

As this process occurs only once, it is sufficient to calculate the mass for any single fragment observed in the mass spectrum. The other fragments will only differ in their mass by the degree of substitution, specifically by [(n - 1) × 58.08] atomic mass units for HP β CD. Thus, given the inability to identify the molecular ion peak

with the highest mass (corresponding to the most highly substituted species), likely due to its exceedingly low intensity (in the region with mass values $> 1,681.05$), we can use the mass of the singly-substituted ion, M^+ (1), as a starting point for calculations:

$$M^+_{m/z(1)} = M + m_{Na} = M_{HP\beta CD(1)} + 23 = 1193,074 + 23 = 1216,074 \text{ Da (see Table 7- Mass spectroscopy } M_1)$$

In the acquired mass spectrum, this fragment, as well as subsequent fragments, are observed. The spectrum also contains peaks corresponding to $[m_H +$

$m_{Na}]^+$ ions or isotopes of carbon or hydrogen produced during the ionization process. Remarkably, a distinguishing characteristic of $HP\beta CD$ in its MS spectrum is the presence of a βCD fragment that does not contain a substituent group, corresponding to a peak with a mass of 1,158.27 Da. To form an ion, in addition to the attack mechanism by Na^+ , the βCD ring has gained an additional Na^+ . It is possible that the βCD molecule does not gain an additional H^+ but rather undergoes a bond cleavage at $-O-C(1)-O-C(4)-$ to form $-O-C(1)-OH$ and $-CH-$. Thus, the mass of this ion will be $M^+_{\beta CD} = M_{\beta CD} + m_{Na^+} = 1,134.994 + 23 = 1,157.994 \text{ Da}$.

Table 7 Mass spectrum of synthesized $HP\beta CD$ in the 1,000 to 2,000 m/z range (calculated according to formula (a)).

M_n	Prediction		Mass spectrum
	$[m_{Na}]^+$	$[m_{Na}]^+$	$[m_H + m_{Na}]^+$ and isotopes
M_1	1,216.074	1,216.26	-
M_2	1,274.154	1,274.32	1,274.91
M_3	1,332.234	1,332.44	1,333.44
M_4	1,390.314	1,390.56	1,391.23
M_5	1,448.394	1,448.55	1,449.29 and 1,449.62
M_6	1,506.474	1,506.74	1,507.48
M_7	1,564.554	1,564.80	-
M_8	1,622.634	1,622.73	-
M_9	1,680.714	-	1,681.05

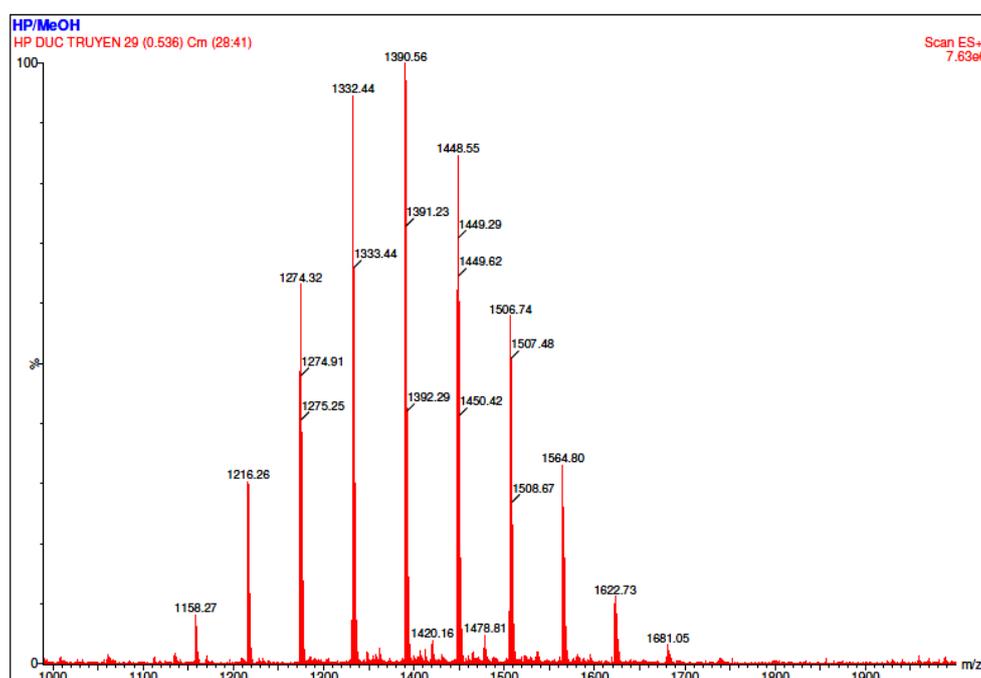


Figure 8 Mass spectrum of the synthesized $HP\beta CD$ in methanol at m/z range of 100 - 2,000.

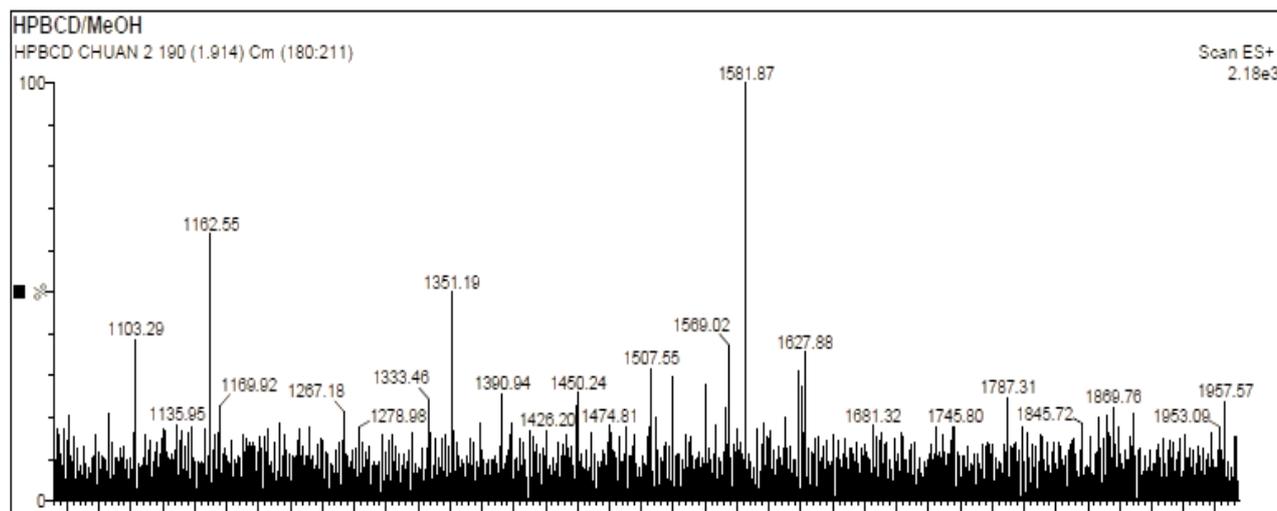


Figure 9 Mass spectrum of the commercial HP β CD measured in methanol at m/z range of 0 - 2,000.

The mass spectra of the synthesized (**Figure 8**) and the commercial HP β CD (**Figure 9**) display distinct ion distribution patterns, reflecting differences in the substitution profile of the hydroxypropyl groups resulting from different synthesis conditions. Commercial HP β CD is an industrial product consisting of a heterogeneous mixture of homologous molecules with a broad degree of substitution ($DS \approx 4 - 6$), leading to a series of evenly spaced molecular ion peaks within the range of approximately 1,300 - 1,600 m/z , corresponding to species with varying numbers of hydroxypropyl substituents. The spectrum appeared as a symmetrical cluster of peaks with relatively consistent intensity, characteristic of a well-mixed product. In contrast, the synthesized HP β CD, prepared under laboratory-scale conditions, exhibited a narrower and slightly shifted distribution of ion clusters. The fewer and less evenly distributed peaks in its spectrum indicated a narrower DS distribution and a distinct substitution pattern, which depended on reaction parameters such as temperature, base concentration, and epoxide-to- β CD molar ratio. The absence of significant peaks below m/z 100 confirmed the exclusion of solvent-related background ions, as the scan range was optimized from 100 - 2,000 m/z for a higher signal-to-noise ratio.

Thus, when HP β CD was synthesized under varying conditions, the substitution patterns also differed. This was evident in the mass spectrum of commercial HP β CD, making direct comparison infeasible. These spectral characteristics demonstrated

that the synthesized HP β CD differs from the commercial one mainly in the distribution and intensity of its molecular ion species. While a direct quantitative comparison is not meaningful due to the polydisperse nature of HP β CD, the differences in ion distribution qualitatively confirm successful derivatization and formation of HP β CD with a distinct substitution profile.

Conclusions

The previous method was proven to be applicable for producing HP β CD on an industrial scale (in kg). This large-scale synthesis of HP β CD formed 1.38 ± 0.05 kg product, yielding $86.77 \pm 0.68\%$, and a degree of substitution of 5.91. The confirmation of the HP β CD structure was re-characterized and additionally compared with the available product in commercial (Roquette, France). As a comparative analysis, the spectroscopic data revealed no significant structural differences between the synthesized HP β CD and a commercially available counterpart. These findings validate the developed synthesis method, highlighting its potential for industrial application and future research into the uses of HP β CD in drug development.

Furthermore, this validation underscores the reproducibility and industrial viability of the developed method. The findings are particularly salient in the Vietnamese context, where the national supply of a critical excipient in drug formulation is now dependent on imports from foreign suppliers, primarily China, India, France, and Japan. Thus, the proven scalability and quality of this domestic synthesis method provide a

strategic pathway for local researchers and chemical manufacturers to achieve critical autonomy in production. This self-sufficiency will enable them to mitigate constraints related to the high costs of commercial products, complex technology transfer procedures, and inherent supply chain instability associated with reliance on foreign sourcing.

Acknowledgements

The authors gratefully acknowledge Hong Bang University and International University for providing materials and laboratory facilities to support this study.

Declaration of generative AI in scientific writing

The authors acknowledge the use of generative AI tools (Google Gemini) in the preparation of this manuscript for language editing and grammar correction. No content generation or data interpretation was performed by AI. The authors take full responsibility for the content and conclusions of this work.

CRedit author statement

Truyen Duc Phung: Conceptualization, Methodology, Supervision, and Formal analysis. **Son Le Hoang:** Supervision, Formal analysis, Data curation, Validation, Visualization, and Writing –original draft. **Thanh Nguyen-Kim Le:** Data curation, Investigation, Visualization, Project administration, and Writing –original draft. **Anh Thi-Phuong Phung:** Data curation, Formal analysis, Investigation, Validation, and Visualization.

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