



Research Article

Synthesis of a cannabidiol-imprinted polymer for selective extraction and HPLC analysis of cannabidiol from *Cannabis sativa*

Wisanu Thongchai^{1*}, Suwanna Thongchai³, Yuttasak Chammui¹, Titiporn Chorchong², Kasama Durongsak² and Sirirat Phanruang¹

¹Chemistry Program, Faculty of Science and Technology, Pibulsongkram Rajabhat University, Phitsanulok 65000, Thailand

²Physics Program, Faculty of Science and Technology, Pibulsongkram Rajabhat University, Phitsanulok 65000, Thailand

³Thathong Phitthayakhom School, Phitsanulok 65000, Thailand

*Corresponding author: wisanuthongchai@psru.ac.th

Received: 27 January 2026; Revised: 30 April 2026; Accepted: 24 June 2026; Published: 30 August 2026

Abstract

A CBD-imprinted polymer (CBD-MIP) was synthesized using UV-initiated photopolymerization. In this procedure, 2-hydroxyethyl methacrylate served as the functional monomer, ethylene glycol dimethacrylate served as the crosslinker, cannabidiol (CBD) served as the template, 2,2-dimethoxy-2-phenylacetophenone as the initiator, with a mixture of acetonitrile and 1-dodecanol as the porogenic solvents. The CBD-MIP was prepared from 0.636 mmol of CBD, 1 mL of acetonitrile, 8.5 mL of 1-dodecanol, 0.229 mmol of 2,2-dimethoxy-2-phenylacetophenone, 23.84 mmol of ethylene glycol dimethacrylate, and 8.22 mmol of 2-hydroxyethyl methacrylate. The molecular interactions between the template and the functional monomers were investigated by examining the adsorption capacity of the CBD-MIPs and the non-imprinted polymers (NIPs). The results indicated that the CBD-MIPs exhibited a higher adsorption efficiency (65%) than the NIPs. Scanning electron microscopy revealed a porous structure containing specific binding cavities, while Fourier-transform infrared spectroscopy confirmed the interactions between CBD and the polymer matrix. A high-performance liquid chromatography (HPLC) method with UV detection was developed and validated under various chromatographic conditions to determine CBD in *Cannabis sativa* samples. The sample solutions were pretreated with the CBD-MIPs by solid-phase extraction and then analyzed on a VertiSep™ Integral C18 column (2.6 μ m, 4.6 $\times$ 150 mm) maintained at 40 °C. The detection wavelength was set at 220 nm, corresponding to the maximum absorbance of CBD. The mobile phase consisted of (A) 0.1% (v/v) phosphoric acid in deionized water and (B) 0.1% (v/v) phosphoric acid in acetonitrile, delivered under gradient elution at a flow rate of 1.2 mL/min, with an injection volume of 5 μ L. The HPLC method showed good sensitivity, with limits of detection (LOD) and quantification (LOQ) of 0.253 and 0.768 μ g/mL, respectively, and recoveries ranging from 86.00% to 98.95%. Application of the method to *Cannabis sativa* samples revealed a higher CBD content in the inflorescences than in the leaves. The proposed method offers a selective and reliable approach for the analysis of CBD in medicinal herbal extracts.

Keywords: CBD-imprinted polymer, high-performance liquid chromatography, solid-phase extraction, selective adsorption, method validation

Introduction

Cannabidiol (CBD), a major terpene phenol compound and a non-psychoactive constituent of *Cannabis sativa*, has received increasing attention due to its reported therapeutic effects, including neuroprotective, antiepileptic, anxiolytic, antipsychotic, anti-inflammatory, analgesic, and anticancer properties [1]. Therefore, the large-scale production of CBD is highly important for the research and development of new drugs. Generally, CBD is prepared through chemical synthesis and plant extraction methods. Compared with chemical synthesis, plant extraction offers several advantages,

including lower cost, higher yield, and a simple preparation procedure. To date, various extraction techniques have been employed, such as ultrasonic-assisted extraction, microwave-assisted extraction, and supercritical fluid extraction. Although the above methods provide bulk CBD primary extracts, several limitations remain, including low selective adsorption for CBD, a long extraction time, a large amount of organic chemical reagent consumption, and complex experimental conditions. Thus, a simple and rapid method is needed to extract and separate CBD from real hemp samples.

Solid-phase extraction (SPE) has been widely used for the selective isolation and enrichment of target analytes. However, its selectivity is often limited by the non-specific nature of conventional sorbents, which leads to the co-extraction of interfering compounds and reduced analytical accuracy. To address these limitations, molecularly imprinted polymers (MIPs) have been widely investigated as selective sorbents because of their ability to form recognition sites tailored to target molecules [2,3-4]. MIPs are prepared by molecular imprinting technology and provide specific recognition and selective adsorption of target molecules (template molecules) and their structural analogues. MIPs offer several advantages: simple process, high selectivity and low operation cost. These materials, often described as synthetic analogues of antibodies, enable selective binding through complementary interactions in size, shape, and functional groups [4-5]. Consequently, MIPs have been successfully applied in solid-phase extraction, sensing, and chromatographic separations, providing improved selectivity compared with non-imprinted counterparts [6-8]. Recent developments, including magnetic and porous MIPs, further demonstrate their potential to improve extraction efficiency and analytical sensitivity [9-10]. Several methods for the separation of cannabidiol using MIP have been reported [11-12]. In addition, the use of photopolymerization to prepare CBD-specific MIPs has received limited attention, and the systematic optimization of monomer-template interactions under these conditions remains insufficiently studied.

In this paper, we describe the synthesis of MIPs for the extraction of cannabidiol and for use as SPE sorbents for the selective extraction of cannabidiol in *Cannabis sativa* extracts. The cannabidiol was used as a template for MIP synthesis using a noncovalent imprinting approach. The polymer was synthesized and characterized for its ability to selectively bind cannabidiol. The CBD-MIPs were synthesized using 2-hydroxyethyl methacrylate (HEMA) as the functional monomer, ethylene glycol dimethacrylate as the crosslinker, acetonitrile and 1-dodecanol as the porogenic solvent, and 2,2-dimethoxy-2-phenylacetophenone as the initiator. HEMA was selected as the functional monomer because of its ability to form hydrogen-bonding interactions with CBD through its hydroxyl group, which may improve the binding affinity compared with methacrylic acid (MAA) and ethyl methacrylate (EMA). Its moderate polarity may also support compatibility with the porogenic solvent and promote the formation of well-defined imprinted sites. The characterization of CBD-MIPs was carried out using Fourier-transform infrared (FT-IR) spectroscopy. The binding affinity of the binding sites in the polymer was assessed using kinetic

adsorption tests. The selectivity for cannabidiol was determined by HPLC with UV detection. The CBD-MIPs method was successfully applied for the extraction of CBD from medicinal plants.

Materials and Methods

Materials and reagents

All chemicals were of analytical grade or higher and were used as received without further purification. Ultrapure water (resistivity $\geq 18.2 \text{ M}\Omega \cdot \text{cm}$ at 298 K) was obtained from a Milli-Q water purification system (Millipore, USA) and was used throughout the experiments. Cannabidiol (CBD, $\geq 99.0\%$ purity) was purchased from Sigma-Aldrich (UK). 2-Hydroxyethyl methacrylate (HEMA, $\geq 99\%$), ethylene glycol dimethacrylate (EGDMA, $\geq 98\%$), 3-(trimethoxysilyl)propyl methacrylate ($\geq 98\%$), and 2,2-dimethoxy-2-phenylacetophenone (DMPAP, $\geq 97\%$) were also purchased from Sigma-Aldrich (UK). Before use, HEMA and EGDMA were passed through a short alumina column to remove the polymerization inhibitors. Acetonitrile (HPLC grade, $\geq 99.9\%$) was used for the chromatographic analysis. Sulfuric acid (H_2SO_4 , 95–97%), sodium hydroxide (NaOH, $\geq 98\%$), and phosphoric acid (H_3PO_4 , 85%) were purchased from Merck (Germany).

Instrumentation

HPLC analyses were carried out on a PerkinElmer Flexar HPLC system (PerkinElmer, Waltham, USA) equipped with a quaternary pump, an autosampler, and a variable-wavelength UV detector, and controlled by the Chromera Chromatography Data System software. Separation was carried out on a VertiSep™ Integral C18 column (2.6 μm , 4.6 $\times$ 150 mm) maintained at 40 °C. A reversed-phase C18 column was selected because of its suitability for separating moderately non-polar compounds such as CBD, providing adequate retention and resolution through hydrophobic interactions. The detection wavelength was set at 220 nm, corresponding to the maximum absorbance of CBD. The mobile phase consisted of (A) 0.1% (v/v) phosphoric acid in deionized water and (B) 0.1% (v/v) phosphoric acid in acetonitrile, delivered under gradient elution at a flow rate of 1.2 mL/min (**Table 1**). The injection volume was 5 μL . System suitability was evaluated before the analysis using replicate injections ($n = 5$) of a standard CBD solution. The parameters assessed included retention time (t_R), peak-area repeatability (%RSD < 2%), theoretical plate number ($N > 2000$), and tailing factor ($T < 2$), which together confirmed adequate system performance for quantitative analysis. Fourier-transform infrared (FTIR) spectra were recorded on a Nicolet iS5 spectrometer (Thermo Fisher Scientific, USA) over the range of 4000–400 cm^{-1} . The morphological characteristics of the synthesized polymers were examined by scanning electron microscopy (SEM).

Table 1. HPLC condition for determination of CBD contents

Gradient Time (min)	Phase A (%)	Phase B (%)
	0.1% H ₃ PO ₄ in Deionized Water	0.1% H ₃ PO ₄ in Acetonitrile
0.1	28	72
5.0	28	72
4.0	5	95
2.0	5	95
0.5	28	72
4.5	28	72

Standard preparation

A stock standard solution of CBD (1,000 µg/mL) was prepared in acetonitrile. The stock solution was stored in amber glass vials at 4 °C, protected from light, and was stable for up to seven days. All standard solutions were equilibrated to room temperature before use. Working standard solutions were freshly prepared by the serial dilution of the stock solution with acetonitrile to obtain the desired concentrations. Calibration standards in the range of 2.5–100.0 µg/mL were prepared daily and analyzed under the optimized HPLC conditions. Each concentration level was analyzed in triplicate ($n = 3$) to ensure the precision of the analysis. The calibration curve was constructed by plotting peak area against CBD concentration, and linear regression analysis was applied to obtain the calibration equation. The method showed good linearity within the studied range, and the calibration model was then used to quantify CBD in real samples.

Extraction procedure

CBD was extracted from *Cannabis sativa* using ethanol as the solvent. Ethanol was selected because of its ability to efficiently dissolve cannabinoids and terpenes while being relatively safe, low-cost, and compatible with downstream analytical techniques. Preliminary experiments were carried out to optimize the extraction conditions, including the solvent type (ethanol and methanol), the solvent-to-solid ratio (3:1–7:1 mL/g), and the extraction time (12–36 h). The optimal conditions were selected based on the extraction yield and CBD content. Under the optimized conditions, *Cannabis sativa* powdered samples (100 g) were mixed with 500 mL of ethanol (solid-to-solvent ratio of 1:5 g/mL) in amber glass bottles. The mixtures were stirred with a magnetic stirrer at room temperature for 24 h. After extraction, the mixtures were filtered and the filtrates were collected. The solvent was removed under reduced pressure at 40 °C on a rotary evaporator, followed by drying in a vacuum oven at 40 °C to obtain the crude extracts. The extracts were weighed to determine the extraction yield and were stored in amber glass vials at –20 °C until analysis (**Figure 1**). The extraction yield was calculated using the following equation: Yield (%) = (mass of dried extract/mass of dried plant material) × 100.

MIPs synthesis

The spherical porous supports were first functionalized to enable the covalent attachment of the polymer layer. The materials were rinsed with acetone and deionized water, followed by activation with 0.2 mol/L NaOH for 30 min to generate surface hydroxyl groups. After washing with ethanol, the supports were silanized in a 20% (v/v) solution of 3-(trimethoxysilyl)propyl methacrylate in 95% ethanol, adjusted to pH 5.0 with acetic acid. The reaction was carried out for 5 h to introduce vinyl groups onto the surface, after which the supports were washed with ethanol and dried under a nitrogen stream [13–15]. The CBD-MIPs were synthesized by UV-initiated free-radical polymerization. The template molecule (CBD, 0.636 mmol) was dissolved in a porogenic solvent mixture of acetonitrile (1.0 mL) and 1-dodecanol (8.5 mL) under continuous stirring in a nitrogen atmosphere to promote the formation of the pre-polymerization complex. DMPAP (0.229 mmol) was used as the photoinitiator because of its ability to generate reactive radical species upon UV irradiation, thereby initiating polymerization under mild conditions. Then, EGDMA (23.84 mmol as the crosslinker and HEMA, 8.22 mmol functional monomer) were added to the reaction mixture. The solution was degassed under vacuum for 15 min to remove dissolved oxygen, which inhibits free-radical polymerization. The mixture was then transferred onto the silanized supports and exposed to UV irradiation at 365 nm for 30 min. The UV source provided an intensity of approximately 10 mW/cm² at a fixed distance of 10 cm from the sample to ensure uniform polymerization. After polymerization, the materials were washed sequentially with acetonitrile and 50% (v/v) methanol–water to remove the template molecule and the unreacted reagents. Washing was continued until no residual CBD was detected in the eluate (monitored by HPLC). The NIPs were prepared under identical conditions without the addition of CBD and were used as the control materials. All polymerizations were carried out in triplicate ($n = 3$) under identical conditions to evaluate the reproducibility. The resulting CBD-MIPs exhibited consistent morphology and adsorption performance, with relative standard deviation (%RSD) values below 5%, confirming the good reproducibility of the synthesis procedure.



Figure 1. Experimental setup for the solid-phase extraction of cannabidiol using CBD-MIPs

Adsorption study

The adsorption performance of the CBD-MIPs was evaluated and compared with that of the NIPs using batch adsorption experiments. A 10 mL aliquot of CBD standard solution (10 µg/mL in acetonitrile) was added to a known amount of polymer, and the mixture was stirred at room temperature under controlled conditions. After incubation, the suspension was separated by filtration, and the residual CBD concentration in the supernatant (C_e) was determined by HPLC. All experiments were performed in triplicate ($n = 3$), and the results were expressed as the mean $\pm$ standard deviation. The adsorption efficiency was calculated using the following equation [11]:

$$\text{Adsorption (\%)} = \frac{(C_0 - C_e)}{C_0} \times 100 \quad (1)$$

where C_0 and C_e (µg/mL) represent the initial and equilibrium concentrations of CBD, respectively.

To investigate the adsorption kinetics, experiments were conducted at different contact times (10–60 min) under identical conditions. The adsorption data were fitted to pseudo-first-order and pseudo-second-order kinetic models to clarify the rate-controlling mechanism and adsorption behavior. Adsorption isotherm studies were carried out using CBD solutions at various concentrations (2.5–10 µg/mL). The equilibrium data were fitted to the Langmuir and the Freundlich isotherm models to evaluate the adsorption capacity and the surface heterogeneity of the polymers. The selectivity of the CBD-MIPs was

assessed by comparing their adsorption performance with that of the NIPs under identical conditions. The imprinting factor (IF) was calculated as [16]:

$$\text{IF} = \frac{Q_{\text{MIP}}}{Q_{\text{NIP}}} \quad (2)$$

where Q (mg/g) represents the adsorption capacity of the polymer. A higher IF value indicates stronger selective recognition of CBD by the imprinted polymer compared with the non-imprinted polymer.

Results and Discussion

Cannabis sativa extraction

The extraction conditions were systematically optimized based on extraction yield and content (Table 2). Among the solvents tested, ethanol provided a higher extraction efficiency than methanol, which is consistent with its intermediate polarity for dissolving both cannabinoids and terpenes [1,11-12]. Under the optimized conditions (ethanol, solid-to-solvent ratio of 1:5 g/mL, 24 h extraction), the extraction yield was $13.6 \pm 0.8\%$ ($n = 3$, %RSD = 5.9). Increasing the solvent ratio from 1:3 to 1:5 significantly improved the extraction yield ($p < 0.05$), whereas a further increase to 1:7 produced no statistically significant improvement, indicating that solvent saturation had been reached. Similarly, the extraction time influenced the yield: extending the duration from 12 h to 24 h increased the yield by approximately 18%, whereas prolonging it to 36 h produced only a marginal improvement (<3%), suggesting that equilibrium had been reached at 24 hours.

Table 2. Extraction yield and optimization

Condition	Yield (%)	SD	%RSD	Reference
1:3 (12 h)	9.8	0.7	7.1	This Study
1:5 (24 h)	13.6	0.8	5.9	
1:7 (36 h)	14.0	0.6	4.3	
Ethanol	12–18			[11]

Polymer synthesis

The CBD-MIPs synthesized by UV photopolymerization exhibited a significantly higher adsorption capacity than the NIPs, confirming successful imprinting. The adsorption efficiency of the CBD-MIPs reached $65.2 \pm 2.8\%$ ($n = 3$), whereas that of the NIPs was only $27.4 \pm 3.1\%$, corresponding to an imprinting factor of approximately 3.0. This quantitative difference indicates the formation of selective binding sites rather than non-specific adsorption [2,4,6]. The porous morphology of the CBD-MIPs, induced by the porogenic solvents (acetonitrile and 1-dodecanol), plays a critical role in their adsorption performance [7]. Phase separation during polymerization generates interconnected pores, which increase the surface area and improve mass transfer. Consequently, the CBD-MIPs exhibit faster adsorption kinetics and higher uptake than the relatively dense structure of the NIPs. This structural feature is consistent with the observed increase in the adsorption efficiency.

At the molecular level, the selective recognition arises from pre-polymerization interactions between CBD and the functional monomer HEMA. Hydrogen bonding between the hydroxyl groups of CBD and HEMA stabilizes the template–monomer complex, leading to the formation of complementary binding cavities after the template is removed. The use of EGDMA as the crosslinker provides structural rigidity, preserving the spatial arrangement and chemical functionality of these cavities. Without such interactions (as in the NIPs), only non-specific binding occurs, which explains the significantly lower adsorption. The photopolymerization process was optimized to ensure the efficient formation of the polymer. Degassing effectively reduced oxygen inhibition, allowing consistent free-radical propagation under UV irradiation (365 nm, 30 min). The reproducibility of the synthesis was confirmed

by the low variability in adsorption performance (%RSD <5%), demonstrating good control over polymer formation and the reliability of the method [3,5].

SEM analysis

SEM was used to examine the surface morphology and structural differences between the CBD-MIPs and the NIPs synthesized by UV photopolymerization (**Figure 2**). At a magnification of 5,000 $\times$, both materials exhibited agglomerated granular structures, which are typical of polymers prepared by free-radical photopolymerization. To provide quantitative support, the pore size and surface characteristics were analyzed from the SEM images using ImageJ software. The CBD-MIPs exhibited a significantly more porous structure, with an average pore diameter of 185 ± 42 nm, compared with 72 ± 25 nm for the NIPs. In addition, the pore density of the CBD-MIPs was estimated at 3.1 pores/ μm^2 , which is approximately 2.4-fold higher than that of the NIPs (1.3 pores/ μm^2). These quantitative differences confirm that the presence of template molecules during polymerization significantly influences the resulting polymer structure. The formation of template–monomer complexes, followed by template removal, generates well-defined cavities, which result in an increased pore size and density.

In contrast, the NIPs lack such templating effects, leading to a denser and more homogeneous morphology. The enhanced porosity and surface roughness of the CBD-MIPs are directly correlated with their improved adsorption performance. Larger and more abundant pores promote mass transfer and the diffusion of target molecules, while the imprinted cavities provide selective binding sites. This structural advantage is consistent with the higher adsorption efficiency and imprinting factor observed for the CBD-MIPs.

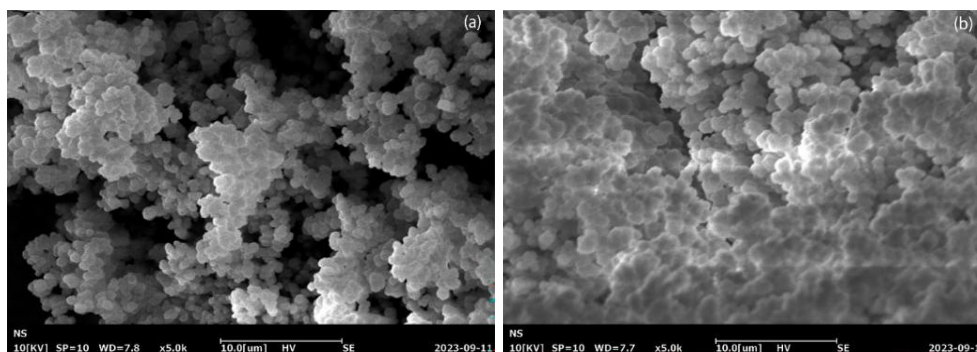


Figure 2. SEM images of (a) CBD-MIPs and (b) NIPs

Table 3. FTIR peak assignment

Wavenumber (cm ⁻¹)	Assignment	Functional Group	Observation (CBD-MIPs vs NIPs)	Reference
3200-3600	O–H stretching	Hydroxyl (HEMA, CBD)	Slightly broader in MIPs	[2,3]
~1719	C=O stretching	Ester (EGDMA)	Minor shift (<10 cm ⁻¹)	[2,3,7]
1450–1600	C=C stretching	Alkene/aromatic	Similar in both	[3]
1000–1300	C–O–C stretching	Ester linkage	No significant shift	[3]
600–900	C–H bending	Aromatic	Slight intensity variation	[3,12]

FTIR analysis

FTIR spectroscopy was used to confirm the chemical structure of the synthesized polymers and to compare the CBD-MIPs with the NIPs (**Figure 3**). Both spectra exhibited characteristic absorption bands corresponding to methacrylate-based polymers, indicating the successful polymerization of HEMA and EGDMA. The major absorption bands observed in both the CBD-MIPs and the NIPs include O–H stretching (3200–3600 cm⁻¹), C=O stretching (~1719 cm⁻¹), and C–O–C stretching (1000–1300 cm⁻¹), which are consistent with previously reported MIP systems based on methacrylate monomers [2,3]. Only minor spectral differences were observed between the CBD-MIPs and the NIPs. A slight broadening and an increased intensity of the O–H stretching band (~3400 cm⁻¹) in the CBD-MIPs may indicate hydrogen-bonding interactions between HEMA and CBD during the pre-polymerization stage.

In addition, a small shift (<10 cm⁻¹) in the C=O stretching region was observed, suggesting the possible involvement of carbonyl groups in weak non-covalent interactions. However, these changes are subtle and should be interpreted with caution, because FTIR alone cannot definitively confirm template–monomer interactions. No new peaks were observed in the CBD-MIPs compared with the NIPs, indicating that no new covalent bonds were formed during imprinting. This finding is consistent with the non-covalent molecular imprinting mechanism widely reported in the literature [4,6]. The bands of the 1000–1300 cm⁻¹ region (C–O and C–O–C stretching) showed comparable positions in both materials, confirming that the polymer backbone structure remained unchanged after imprinting [8]. Slight intensity variations may be attributed to differences in polymer packing or local environment rather than to definitive structural changes [3,5].

Adsorption performance

The adsorption performance of the CBD-MIPs synthesized using different functional monomers

was evaluated and compared with that of their corresponding non-imprinted polymers (NIPs), as presented in **Figure 4**. As shown, the HEMA-based MIP exhibited the highest adsorption efficiency (65%), which was significantly higher than that of its corresponding NIP (5%; $p < 0.05$). In contrast, the MAA- and EMA-based MIPs showed considerably lower adsorption efficiencies (<10%), indicating a weaker affinity towards CBD molecules. The enhanced performance of the HEMA-MIP can be attributed to the presence of hydroxyl functional groups, which promote hydrogen bonding and polar interactions with CBD. These interactions are likely to support the formation of stable template–monomer complexes during polymerization, resulting in more effective imprinting [3,6-7].

Similar observations have been reported in previous studies, in which hydroxyl-containing monomers improved the binding affinity towards cannabinoid compounds. The adsorption capacity (Q) of the optimized HEMA-MIP was determined to be 11.0 ± 0.8 mg/g, which was significantly higher than that of the NIP (4.0 ± 0.6 mg/g; $p < 0.05$). This result confirms the successful formation of selective binding sites within the polymer matrix. The imprinting factor (3.0) further indicates effective molecular imprinting and enhanced selectivity. Compared with previously reported materials [12], the adsorption capacity obtained in this study falls within the reported range for CBD-MIPs (10 mg/g) and is slightly higher than that of some conventional systems. Although magnetic MIPs exhibit a higher adsorption capacity (26.5 mg/g), the HEMA-MIP developed in this work offers the advantages of a simpler synthesis and adequate adsorption performance. The observed adsorption behavior can be attributed to the formation of imprinted cavities that are complementary in size, shape, and functional-group orientation to CBD molecules. This observation is consistent with the molecular recognition mechanism typically described for MIPs.

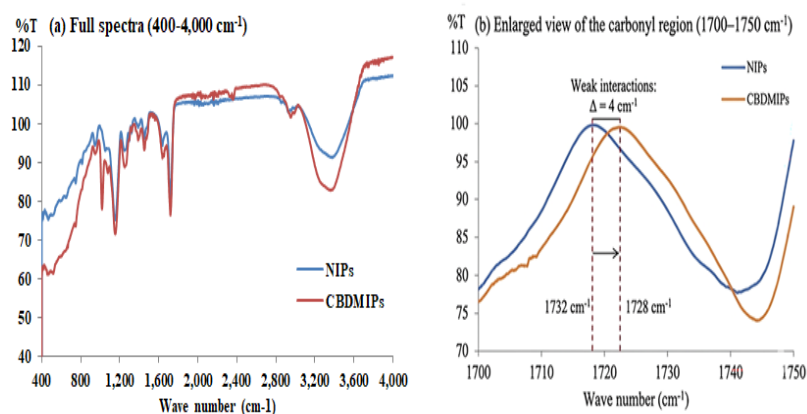


Figure 3. FTIR spectra of the CBD-MIPs and NIPs. (a) Full spectra (400–4000 cm^{-1}) and (b) enlarged view of the carbonyl region (1700–1750 cm^{-1}), showing a slight shift in the C=O stretching band of the CBD-MIPs compared with the NIPs, suggesting weak interactions between the template and the functional monomer

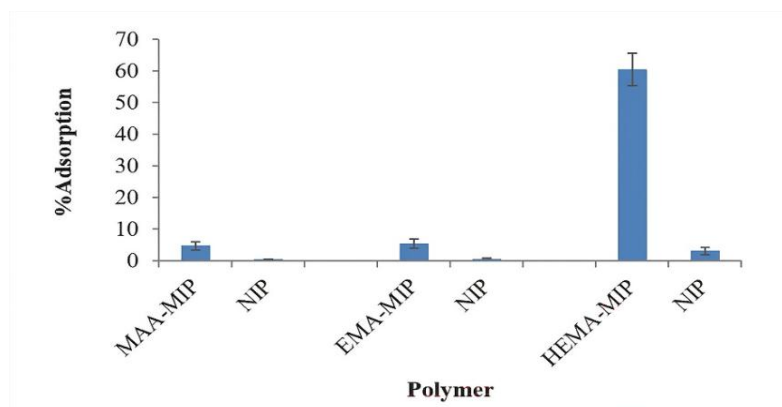


Figure 4. Adsorption percentage of cannabidiol on MAA-MIP, EMA-MIP, HEMA-MIP, and corresponding NIPs

Method validation

The analytical performance of the proposed CBD-MIPs method was validated in terms of linearity, sensitivity, accuracy, precision, selectivity, and repeatability.

Linearity and calibration range

Under the optimized chromatographic conditions, the linearity of the method was evaluated using CBD standard solutions. To better represent real-sample variability, the calibration range was extended to 2.5–100.0 $\mu\text{g/mL}$. The calibration curves were constructed by plotting the peak area against the CBD concentration. A good linear relationship was obtained from the regression equation, $y = 1976x + 25736$ ($r^2 = 0.9986$), indicating excellent linearity within the studied range.

Limit of detection (LOD) and limit of quantification (LOQ)

The LOD and LOQ were determined from the standard deviation of the response (SD) and the slope of the calibration curve (S). The SD was

obtained from replicate measurements of the blank samples ($n = 10$). The following equations were applied: $\text{LOD} = 3.3(\text{SD}/S)$ and $\text{LOQ} = 10(\text{SD}/S)$. The LOD and LOQ values were 0.253 $\mu\text{g/mL}$ and 0.768 $\mu\text{g/mL}$, respectively, indicating adequate sensitivity for CBD determination.

Accuracy

Accuracy was assessed by recovery studies at three concentration levels (5, 10, and 15 $\mu\text{g/mL}$). The percentage recoveries ranged from 86.00% to 98.95%, which is within the acceptable range (80–120%). A slight decrease in recovery at the higher concentrations (15 $\mu\text{g/mL}$) was observed, possibly because of partial saturation of the binding sites or reduced extraction efficiency.

Precision and repeatability

Precision was assessed in terms of intra-day (repeatability) and inter-day precision at three concentration levels (5, 10, and 15 $\mu\text{g/mL}$). The results are summarized in **Table 4**. For intra-day precision, the measured concentrations were 4.90,

9.38, and 13.36 $\mu\text{g/mL}$, corresponding to recoveries of 98.01%, 93.80%, and 89.09%, respectively. For inter-day precision, the measured concentrations were 4.94, 9.46, and 12.90 $\mu\text{g/mL}$, with recoveries of 98.95%, 94.66%, and 86.00%, respectively. The low RSD values ($< 5\%$) indicate good repeatability and acceptable intermediate precision of the method. A slightly higher variation at 15 $\mu\text{g/mL}$ may be attributed to reduced extraction efficiency at the higher analyte concentrations.

Analysis of CBD using CBDMIPs by HPLC

Chromatographic analysis of CBD was carried out under the optimized HPLC conditions, and the performance of the CBD-MIPs was compared with that of the NIPs. CBD eluted at a retention time of approximately 12–13 min as a well-defined, symmetrical peak (**Figure 5**). The chromatographic performance was evaluated using key system-suitability parameters. The tailing factor (T) for CBD was ≤ 1.2 , indicating good peak symmetry, while the number of theoretical plates (N) exceeded 2000, confirming adequate column efficiency. The resolution (R_s) between CBD and the adjacent peaks was greater than 1.5, confirming satisfactory separation from potential interfering compounds. The chromatograms obtained using the CBD-MIPs showed a significantly higher peak intensity than those obtained with the NIPs at the CBD retention time. This finding indicates an enhanced extraction efficiency and a stronger binding affinity of the imprinted polymer towards CBD. The increased peak area is attributed to the presence of specific recognition sites formed during the imprinting process, which are complementary to CBD in size, shape, and functional-group orientation. In contrast,

the NIPs exhibited a lower peak intensity and broader peaks, reflecting weaker interactions and higher non-specific adsorption. In addition, the CBD-MIPs chromatograms displayed fewer interfering peaks, indicating improved selectivity and a cleaner extraction. These results confirm that the molecular imprinting process successfully generated selective binding sites, which improved the chromatographic performance, including better peak shape, higher sensitivity, and enhanced selectivity. The validated method was applied to determine the CBD content in different hemp plant samples. The results are presented in Table 5. The CBD concentrations varied significantly among the plant organs. The inflorescences consistently contained a higher CBD content than the leaves. This observation is consistent with previous reports indicating that cannabinoid biosynthesis occurs primarily in the glandular trichomes, which are more abundant in the inflorescences.

Among the cultivars analyzed, Northern Light exhibited the highest CBD levels in both the leaves (6.15 ± 0.12 mg/g) and inflorescences (32.07 ± 1.45 mg/g). In contrast, CBD was not detected in the leaves of the CBF1 and Auto Blue, whereas their inflorescences contained lower CBD levels (4.83 ± 0.57 mg/g and 1.44 ± 0.13 mg/g, respectively). This observation is consistent with the findings of Happyana et al., who reported that inflorescences are the primary sites for cannabinoid biosynthesis and accumulation owing to their higher density of glandular trichomes compared with other plant tissues [12]. These findings suggest that genetic variation significantly influences CBD biosynthesis and accumulation in the *Cannabis sativa* plants.

Table 4. Results of precision and repeatability test

Concentration ($\mu\text{g/mL}$)	Intra-day Found ($\mu\text{g/mL}$)	Recovery (%)	Inter-day Found ($\mu\text{g/mL}$)	Recovery (%)	RSD (%)
5	4.90	98.01	4.94	98.95	0.58
10	9.38	93.80	9.46	94.66	0.60
15	13.36	89.09	12.90	86.00	2.48

Table 5. Cannabidiol content from *Cannabis sativa* leaves and inflorescences

<i>Cannabis sativa</i> Sample	Cannabidiol Content (mg/g)	
	<i>Cannabis sativa</i> Leaves	<i>Cannabis sativa</i> Inflorescences
Northern Light	6.15 ± 0.12	32.07 ± 1.45
CBF1	Not detected	4.83 ± 0.57
Auto Blue	Not detected	1.44 ± 0.13

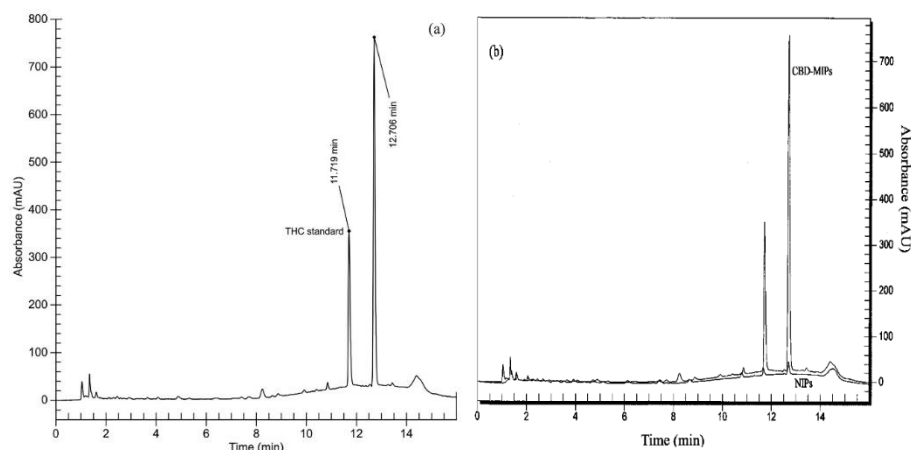


Figure 5. (a) Chromatograms of the THC and CBD standard solution and (b) chromatograms of THC and CBD eluting with CBD-MIPs and NIPs

Conclusion

In this study, CBD-MIPs for the selective extraction and quantification of cannabidiol were successfully synthesized via UV-induced photopolymerization. The prepared CBD-MIPs exhibited distinct morphological and chemical characteristics compared with non-imprinted polymers, with a porous structure and well-defined binding cavities that were complementary to CBD molecules. The use of HEMA as the functional monomer produced the highest adsorption efficiency (65%) and satisfactory polymerization performance. The optimized CBD-MIPs showed an adsorption capacity of 11 mg/g, which was significantly higher than that of the corresponding NIPs, confirming the effectiveness of the imprinting process. The developed HPLC method showed good analytical performance, with acceptable linearity, good sensitivity (LOD: 0.253 $\mu\text{g/mL}$; LOQ: 0.768 $\mu\text{g/mL}$), good accuracy (86–98.95%), and good precision (RSD < 5%). Application to *Cannabis sativa* samples revealed that CBD accumulates predominantly in the inflorescences, consistent with the known patterns of cannabinoid biosynthesis.

From an analytical perspective, this work demonstrates the potential of CBD-MIPs as selective sorbents for the sample preparation and quantification of cannabinoids, offering advantages such as improved selectivity, reduced interference, and a relatively simple synthesis compared with more complex materials (for example, magnetic MIPs). This approach offers a promising strategy for developing cost-effective and reliable analytical methods for cannabinoid analysis in *Cannabis sativa* plant matrices. However, several limitations of this study should be acknowledged. The adsorption capacity of the developed MIPs is lower than that of some recently reported advanced materials, and the calibration range remains relatively narrow for highly variable real samples. In addition, the select-

ivity was evaluated against a limited number of potential interfering compounds, and large-scale applicability has not yet been established. Future work should focus on improving the adsorption capacity through the optimization of polymer composition and porosity, extending the validation to a wider range of matrices and interferents, and comparing performance with commercial SPE sorbents. The integration of CBD-MIPs into automated or online extraction systems may further extend their applicability to routine analytical workflows.

Acknowledgement

We would like to express our sincere thanks to the Program of Chemistry, Faculty of Science and Technology, Pibulsongkram Rajabhat University, for partial support of this work.

References

1. Suzaei, F. M., Daryanavard, S. M., Abdel Rehim, A., Bassyouni, F. and Abdel Rehim, M. (2022). Recent molecularly imprinted polymers applications in bioanalysis. *Chemical Papers*, 1-37.
2. Alexander, C., Andersson H. S., Andersson, L. I., Ansell, R. J., Kirsch, N., Nicholls, I. A., O'Mahony, J. and Whitcombe, M. J. (2006). Molecular imprinting science and technology: A survey of the literature for the years up to and including. *Journal of Molecular Recognition*, 19 (2): 106 – 180.
3. Chen, L., Wang, X., Lu, W., Wu, X. and Li, J. (2016). Molecular imprinting: perspectives and applications. *Chemical Society Reviews*, 45: 2137 – 2211.
4. Haupt, K. and Mosbach, K. (2000). Molecularly imprinted polymers and their use in biomimetic sensors. *Chemical Reviews*, 100(7): 2495 – 2504.
5. Wulff, G. (1995). Molecular imprinting in

- cross-linked materials with the aid of molecular templates-A way towards artificial antibodies. *Angewandte Chemie International Edition*, 34 (17): 1812 – 1832.
6. Saylan, Y., Akgönüllü, S., Yavuz, H., Ünal, S. and Denizli, A. (2019). Molecularly imprinted polymer-based sensors for medical applications. *Sensors*, 19 (6): 1279.
7. Caro, E., Marcé, R. M., Borrull, F., Cormack, P. A. G. and Sherrington, D. C. (2006). Application of molecularly imprinted polymers to solid-phase extraction of compounds from environmental and biological samples. *Trends in Analytical Chemistry*, 25 (2): 143 – 154.
8. Jayasinghe, G. D. T. M. and Moreda-Piñeiro, A. (2021). Molecularly Imprinted Polymers for Dispersive (Micro)Solid Phase Extraction: A Review. *Separations*, 8 (7): 99.
9. Yimin, R., Aimaiti, Z., Tursun, E. and Tuerxun, M. (2025). Magnetic molecularly imprinted polymers for efficient solid-phase extraction and HPLC analysis of myricetin in pomegranate pomace. *RSC Advances*, 15: 25425 – 25435.
10. Moriya, D. H., de Souza Teles, C. F., Mantovani dos Santos, P., Wong, A. and Sotomayor, M. D. P. T. (2025). Hollow porous molecularly imprinted polymer for xylose detection in sugarcane bagasse by dispersive solid-phase extraction. *Materials Advances*, 6: 5901 – 5913.
11. Yang, F., Fu, D., Li, P., Sui, X., Xie, Y., Chi, J., Liu, J. and Huang, B. (2022). Magnetic molecularly imprinted polymers for the separation and enrichment of cannabidiol from Hemp leaf samples. *ACS Omega*, 8: 1240 – 1248.
12. Happyana, N., Agnolet, S., Muntendam, R., van Dam, A., Schneider, B. and Kayser, O. (2013). Analysis of cannabinoids in Laser-microdissected trichomes of medicinal Cannabis sativa using LCMS and cryogenic NMR. *Phytochemistry*, 87: 51 – 59.
13. Thongchai, W., Liawruangrath, B., Liawruangrath, S. and Greenway, G. M. (2014). A microflow chemiluminescence system for determination of chloramphenicol in honey with preconcentration using a molecularly imprinted polymer. *Talanta*, 81 (1-2): 65 – 70.
14. Thongchai, W. and Fukngoen, P. (2018). Synthesis of curcuminoid-imprinted polymers applied to the solid-phase extraction of curcuminoids from turmeric samples. *Journal of Pharmaceutical Analysis*, 8 (1): 46 – 53.
15. Thongchai, W., Poolprasert, P. and Thongchai, S. (2021). The synthesis of molecularly imprinted polymers on microcentrifuge tube filters for solid-phase extraction and the HPLC-UV determination of andrographolides. *Journal of Chromatographic Science*, 59(1): 68 – 76.
16. Song, L., Zhang, J., Zhang, X., Rui, C., Zhang, Y., Wang, M. and He, J. (2023). Study on synthesis and properties of surface molecularly imprinted polymer based on HKUST-1 for adsorption of aflatoxins. *Iranian Polymer Journal*, 32: 1229 – 1240.