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Original Research Article

Synthesis of curcuminoid-imprinted polymers applied to the solid-phase extraction of curcuminoids from turmeric samples

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ABSTRACT

A molecular imprinting polymer technique was successfully applied to precipitation polymerization by using styrene as a functional monomer, curcuminoids as templates, acetonitrile as a porogenic solvent, benzoyl peroxide as the initiator, and ethylene glycol dimethacrylate as the crosslinker. The effects of interaction on the adsorption capacity of the molecularly imprinted polymer (MIP) and non-imprinted polymer (NIP) were investigated. A comparison of the adsorption capacity for MIP and NIP indicated that the NIP had the lowest adsorption capacity. The curcuminoid-imprinted polymer (Cur-MIP) was synthesized from 0.0237 mmol of styrene, 47.0 g of acetonitrile, 1.0238 mmol of ethylene glycol dimethacrylate, 0.0325 mmol of curcuminoids, and 0.2480 mmol of benzoyl peroxide. A high-performance liquid chromatography method with fluorescence detection was developed and validated for various chromatographic conditions for the determination of the curcuminoids in turmeric samples. The sample solution was separated using the Cur-MIP via solid-phase extraction and analyzed on a Brownlee analytical C_{18} column (150 mm × 6 mm, 5 μm) using an isocratic elution consisting of acetonitrile and 0.1% trichloroacetic acid (40:60, v/v). The flow rate was maintained at 1.5 mL/min. The fluorescence detector was set to monitor at λ_{ex} = 426 nm and λ_{em} = 539 nm. The quantification limit values were found to be 16.66, 66.66, and 33.33 μg/L for curcumin, demethoxycurcumin, and bisdemethoxycurcumin, respectively. Thus, we concluded that the Cur-MIP and high-performance liquid chromatographic-fluorescence method could be applied to selective extraction and could be used as a rapid tool for the determination of curcuminoids in medicinal herbal extracts.

1. Introduction

The rhizome of turmeric, *Curcuma longa* L. (Zingiberaceae), has long been used as an orange–yellow coloring agent. This plant is considered as a rich source of phenolic compounds, the so-called curcuminoids. Curcumin contain three different diarylheptanoids: curcumin (CUR), demethoxycurcumin (DMC), and bisdemethoxycurcumin (BDMC). Curcuminoids are pharmacologically significant compounds with strong antioxidant, anti-inflammatory, antibacterial, antiparasitic, antimutagenic, and anticarcinogenic activities [1]. The pharmaceutical use of curcuminoids is gaining popularity. For example, curcuminoids are currently used in the formulation of some sunscreens. These compounds might have antioxidant and skin-lightening properties and could be used to treat skin inflammations, thus making these compounds useful in cosmetic formulations [2]. Numerous analytical methods have been reported by some researchers for the determination of curcuminoids. Flow injection analysis methods include spectrophotometric and fluorometric methods, which express the total color content of the sample [2,3]. However, spectrophotometric methods cannot be used to separate and quantify the curcuminoids individually. High-performance thin layer chromatography (HPTLC) and high-performance liquid chromatography (HPLC) are the methods of choices for the determination of curcuminoids because of the high precision, accuracy, and low detection limit of these methods. The main disadvantages of liquid chromatographic methods, however, are their lower separation efficiencies, expensive columns, and the production of large amounts of organic waste. Hence, new approaches for the rapid identification and quantification of curcuminoids in various matrices are required. Furthermore, the separation power must be increased using solid-phase extraction (SPE), and multiple development in thin layer chromatography before analysis with the HPLC method [4–6]. SPE is an extraction technique based on the selective partitioning of one or more compounds between two phases. This technique has become widely used for sample pretreatment because it is easy, automated, flexible, and environmentally friendly [7]. The SPE

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technique can be performed off-line, where the sample preparation is separated from the subsequent chromatographic analysis, or on-line by direct connection to the chromatographic system.

A molecular imprinting technology was first reported by Wulff and Sarhan [8]. This technique allows the introduction of molecular recognition properties to a functional polymer that is synthesized in the presence of the template molecules. Recently, research interest in molecularly imprinted polymers (MIPs) has increased significantly, along with their potential applications. Applications of MIPs range from SPE materials to receptors in sensors and immuno-like assays [9–14]. MIPs have several advantages such as low cost, ease of production, and excellent physical and chemical stability [15]. MIPs have been widely studied as separation media for liquid chromatographic separation and SPE, replacing the typical affinity gels based on biomolecules [16]. A variety of methods for the separation of the curcuminoids using MIP has been reported [17–23].

In this paper, we describe the synthesis of MIPs for the extraction of curcuminoids and for use as SPE sorbents for the selective extraction of curcuminoids in turmeric extracts and pharmaceutical products. The curcuminoid was used as a template for MIP synthesis using a noncovalent imprinting approach. The polymer was synthesized and characterized for the ability of the polymer to selectivity bind curcuminoids. The curcuminoid-molecularly imprinted polymer (Cur-MIP) was synthesized using styrene as the functional monomer, ethylene glycol dimethacrylate as the crosslinker, acetonitrile as the porogenic solvent, and benzoyl peroxide as the initiator. The characterization of Cur-MIP 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 curcuminoids was determined by HPLC with fluorescence detection. The Cur-MIP method was successfully applied for the extraction of curcuminoids from medicinal plants, pharmaceutical products, and biological samples.

2. Experimental

2.1. Materials and reagents

All chemicals used were of analytical reagent grade. Deionized distilled water was used throughout the experiment (Milli-Q water purification system, Millipore Co., USA). A curcuminoid standard sample (purity: 99%) was purchased from Wako Pure Chemical, Osaka, Japan. Ethyl methacrylate (EMA), styrene, methacrylic acid (MAA), ethylene glycol dimethacrylate (EGDMA), and benzoyl peroxide (BPO) were purchased from Sigma–Aldrich (United Kingdom). Tetrahydrofuran (THF), trichloroacetic acid (TCA), sulfuric acid, ethanol, and toluene were purchased from Aldrich (Poole, Dorset, United Kingdom). Methanol and acetonitrile (HPLC grade) were purchased from E. Merck (Darmstadt, Germany).

2.2. Instruments and HPLC analysis

HPLC-fluorescence detection was performed on a PerkinElmer® Flexar® HPLC system (PerkinElmer Flexar®, Waltham, USA) with a quaternary analytical pump, variable wavelength detector, auto-injector sampler, and Chromera® Chromatography Data Systems (CDS) software. The emission and excitation wavelengths of the fluorescence detector were λ_{ex} = 426 nm and λ_{em} = 539 nm for the determination of curcuminoid concentration in the adsorption capacity study. All analyses were carried out on Brownlee Analytical C_{18} column (150 mm × 6 mm, 5 μm; PerkinElmer) operated at a temperature of 30 °C. The mobile phase was an isocratic elution consisting of acetonitrile and 0.1% TCA (40:60, v/v). The flow rate was maintained at 1.5 mL/min, and the sample injection volume was set to 5 μL. A Soxhlet apparatus was used for template removal from the synthesized MIPs. For spectroscopic characterization, a Thermo Scientific (Nicolet IS5) FT-IR spectroscopy was employed. The surface morphology was analyzed using scanning electron microscopy (SEM) with an electron microprobe JXA 840 A (Jeol, Japan) combined with the LINK analytical system.

2.3. Preparation of standard solutions

A stock standard solution of curcuminoids (1000 mg/L) was freshly prepared by dissolving 25 mg of curcuminoid standards (accurately weighed) in acetonitrile, followed by making the solution up to 25 mL with acetonitrile. Working standard solutions of the curcuminoids (0.1–50 mg/L) were prepared by the appropriate serial dilution of a stock standard solution of curcuminoids in acetonitrile.

2.4. Calibration curve of curcuminoids

Different concentrations of the standard stock solution (0.1, 0.5, 1, 2, 3, 4, 5, 10, 20, 30, 40, and 50 mg/L) were analyzed by the HPLC-fluorescence method. The peak area versus curcuminoid concentration was analyzed by linear-least squares regression, and the regression equation was obtained from the standard curve and used to estimate the concentration of curcuminoids in different samples.

2.5. Sample pretreatment

2.5.1. Fresh turmeric extract

Fresh turmeric samples were purchased from a commercial market in Phitsanulok province, Thailand. The samples were labeled according to their origin, i.e., turmeric 1 and turmeric 2 were purchased from Watyai market and turmeric 3 was purchased from Kokmatoom market. A voucher specimen of the plant was deposited in the herbarium of the Faculty of Pharmacy, Chiang Mai University, Chiang Mai Province, Thailand for reference. The samples were washed and dried in a hot air oven at 50 °C for 36 h. The dried material was ground to a fine powder, passed through a 60-mesh sieve, and kept in an airtight container at 4 °C until further use. Around 1 kg of turmeric root was chopped into small pieces and dried in a hot air oven at 50 °C for 36 h. Then, the dried turmeric was powdered and about 0.5 g was accurately weighed, extracted with 10 mL of ethanol, and sonicated for 30 min, followed by centrifugation for 15 min at 2000 rpm. The supernatant was evaporated to dryness at 60 °C using a rotary evaporator. Then, the residue was reconstituted in 1 mL of ethanol. This solution was used for separation by Cur-MIP and NIP packed in a syringe. After separation, the organic layer was dried under a stream of nitrogen gas using a low heat setting. The extracted dried product was resuspended in 10 mL of acetonitrile. An aliquot of this solution was filtered through a 0.45-μm nylon membrane. Then, 5 μL of this solution was injected into the HPLC-fluorescence system.

2.5.2. Pharmaceutical capsules

Pharmaceutical capsule products (capsules 1, capsules 2, and capsules 3) were purchased from a commercial drug store in Phitsanulok Province, Thailand. A 0.5-g sample was accurately weighed, extracted with 10 mL of ethanol, and sonicated for 30 min, followed by centrifugation for 15 min at 2000 rpm. The supernatant was evaporated to dryness at 60 °C using a rotary evaporator. Then, the residue was reconstituted in 1 mL of ethanol. This solution was used for separation using Cur-MIP and NIP packed in a syringe.

After separation, this organic layer was dried under a stream of nitrogen gas using a low heat setting, and the extracted dried product was resuspended in 10 mL of acetonitrile. An aliquot of this solution was filtered through a 0.45-μm nylon membrane. Then, 5 μL of this solution was injected into the HPLC-fluorescence system.

2.5.3. Curcuminoid-spiked urine sample

A urine sample was spiked with a standard curcuminoid sample and used for testing. This process began with the following modified procedure of Dennis [24]. Briefly, 200 μL of the curcuminoid standard and sample were aseptically transferred using a micropipette into 2 mL microcentrifuge tubes. In each tube, 80 μL deionized water and a curcuminoid standard solution (0, 0.1, 0.5, or 1.0 mg/L) were added. Then, the sample was reconstituted in 1 mL of ethanol. The tubes were capped and mixed for 2 min at medium speed setting by a vortex mixer. This solution was used for separation by Cur-MIP and NIP, which were packed into a syringe. After separation, the supernatant liquid was transferred into a 10-mL volumetric flask and made up to this volume with acetonitrile. An aliquot of this solution was filtered through a 0.45-μm nylon membrane. Then, 5 μL of this solution was injected into the HPLC-fluorescence system.

2.6. Preparation of curcuminoid-imprinted and non-imprinted polymers by precipitation polymerization

A pre-polymerization solution consisting of 0.0325 mmol of curcuminoids, 2.40 g of functional monomers (0.0237 mmol styrene, 0.0280 mmol MAA, and 0.0211 mmol EMA), 1.0238 mmol of EGDMA, 0.2480 mmol of BPO, and 47.0 g of acetonitrile was prepared in a screw-capped glass vial. The reaction molar ratio of functional monomer, template molecule, and crosslinker for the preparation of MIPs was 1:4:130. The solution was sonicated for 20 min and then purged with a stream of nitrogen for 10 min. The Cur-MIP was placed in a water bath and kept at 70 °C for 2 h for polymerization. The resulting bulk polymers were filtered and dried at 70 °C for 1 h. Subsequently, the Cur-MIP was then washed with ethanol, followed by 10% sulfuric acid in ethanol, to remove the template and residues of nonreactive species. The Soxhlet apparatus was used for template removal from the synthesized MIP. The NIP particles were prepared simultaneously under the same conditions without the addition of the template. A similar procedure was used for the synthesis of different MIPs of curcuminoids with various monomers (styrene, MAA, and EMA) and different compositions of porogen for precipitation polymerization. The different MIPs were denoted by the monomer used (Table 1). The subscript number indicates the different porogen content, i.e., EMA_1, MAA_1, and $Styrene_1$; EMA_2, MAA_2, and $Styrene_2$; and EMA_3, MAA_3, and $Styrene_3$.

2.7. Polymer characterization

The structures of the Cur-MIP and NIP samples were characterized by FT-IR spectroscopy. The analysis was performed between 500 and 4000 cm^{-1} at a resolution of 2 cm^{-1}. The surface morphology was analyzed by SEM.

2.8. Adsorption experiment

To a series of ten 100-mL conical flasks containing 0.5 g of the MIPs (EMA_1, MAA_1, and $Styrene_1$; EMA_2, MAA_2, and $Styrene_2$; and EMA_3, MAA_3, and $Styrene_3$) and NIP beads, 25 mL of acetonitrile containing 10 mg/L of curcuminoids was added. The conical flasks were shaken on the shaker at 100 rpm, and the samples were collected after 24 h. The collected samples were centrifuged at 4000 rpm for 20 min. After adsorption, the concentrations of curcuminoids were recorded by using the HPLC-fluorescence system. The adsorption capacity of MIPs and NIP of curcuminoids was calculated using Eq. (1).

$$\text{Adsorption capacity} = (C_1 \times C_2)V/W_{(g)} \quad (1)$$

Here, C_1 and C_2 are the concentration of curcuminoids before and after the adsorption experiments, respectively, V is the volume of the solution containing the curcuminoids, and $W_{(g)}$ is the weight of the polymer particles.

In the adsorption kinetic tests, to a series of 100-mL conical flasks containing 0.5 g of the MIP (Cur_1-Cur_{12}) and NIP polymer beads (Table 1), 25 mL of acetonitrile containing 10 mg/L of curcuminoids was added. The conical flasks were shaken on the

Table 1
The effect of the type and amount of functional monomer, and porogenic solvent used in the preparation of MIP and NIP.

Polymer code	Functional monomer (mmol)			Initiator; BPO (g)	Cross linker; EGDMA (mmol)	Porogenic solvent (g)		Curcuminoids (mmol)
	Styrene	MAA	EMA			Acetonitrile	THF	
EMA_1	–	–	0.0211	0.2480	1.0238	47.0	–	0.0325
MAA_1	–	0.0280	–	0.2480	1.0238	47.0	–	0.0325
$Styrene_1$	0.0237	–	–	0.2480	1.0238	47.0	–	0.0325
EMA_2	–	–	0.0211	0.2480	1.0238	–	47.0	0.0325
MAA_2	–	0.0280	–	0.2480	1.0238	–	47.0	0.0325
$Styrene_2$	0.0237	–	–	0.2480	1.0238	–	47.0	0.0325
EMA_3	–	–	0.0211	0.2480	1.0238	23.5	23.5	0.0325
MAA_3	–	0.0280	–	0.2480	1.0238	23.5	23.5	0.0325
$Styrene_3$	0.0237	–	–	0.2480	1.0238	23.5	23.5	0.0325
Cur_1	0.0237	–	–	0.2480	1.0238	47.0	–	0.0650
Cur_2	0.0237	–	–	0.2480	1.0238	23.5	–	0.0650
Cur_3	0.0237	–	–	0.2480	0.5119	47.0	–	0.0650
Cur_4	0.0237	–	–	0.2480	0.5119	23.5	–	0.0650
Cur_5	0.0237	–	–	0.2480	1.0238	47.0	–	0.0325
Cur_6	0.0237	–	–	0.2480	1.0238	23.5	–	0.0325
Cur_7	0.0237	–	–	0.2480	0.5119	47.0	–	0.0325
Cur_8	0.0237	–	–	0.2480	0.5119	23.5	–	0.0325
Cur_9	0.0237	–	–	0.2480	1.0238	47.0	–	0.0163
Cur_{10}	0.0237	–	–	0.2480	1.0238	23.5	–	0.0163
Cur_{11}	0.0237	–	–	0.2480	0.5119	47.0	–	0.0163
Cur_{12}	0.0237	–	–	0.2480	0.5119	23.5	–	0.0163
NIP	0.0237	–	–	0.2480	1.0238	47.0	–	–

shaker at 100 rpm at 40 °C, and samples were collected at different time intervals (0, 30, 60, 90, 120, 150, 240, 360, and 720 min). The collected samples were centrifuged at 4000 rpm for 20 min to remove any suspended particles, and the supernatant was used for further analysis. For analysis, all samples were filtered through a 0.45-μm filter membrane to minimize the interference of particles during analysis. The concentrations of the curcuminoids after adsorption were analyzed and recorded using HPLC-fluorescence detection. The adsorption kinetic curve of NIP was carried out by the same procedure as MIPs. The adsorption kinetics of MIP and NIP of curcuminoids were calculated using Eq. (2).

$$\textit{Binding capacity} = (C_a \times C_b)100/C_a \quad (2)$$

Here, C_a is the initial curcuminoids concentration in the solution, and C_b is the final curcuminoid concentration in the solution.

2.9. Preparation of SPE cartridges using the Cur-MIP and NIP as the sorbents

The Cur-MIP and NIP powders were ground in a mortar and sieved. Then, the polymer was packed in a syringe (syringe tube volume 3 mL). In extraction steps to establish the optimum conditions under which the analyte could be recognized by the corresponding MIP, a standard solution of curcuminoids was prepared in a mixture of acetonitrile and 0.1% TCA. To a 3 mL syringe tube, 0.5 g of Cur-MIP and NIP were packed. Before analyte loading, the polymer sample was preconditioned with 3 mL of ethanol, 3 mL of acetonitrile, and 3 mL of 0.1% TCA, respectively. The SPE tests were performed in four steps. The first step was the conditioning of the material with deionized water over 24 h. Then, the solvent was removed by a vacuum pump connected to the SPE system. Thereafter, in the loading step, 0.05 mL of each solution (10 mg/L) was used. As a reference, a small amount of each sample was analyzed using the UV spectrophotometer before being added to the polymer. Then, the solution was placed in the column and was removed, drop by drop, into a flask for analysis. The absorbance of each collected solution was measured by UV spectrophotometer. The fraction of curcuminoids retained in the polymer was then calculated. In the third step, the polymer was washed with 3 mL of toluene and 0.1% TCA (50:50, v/v), which were run through the syringe. In the elution step, the curcuminoids retained in the polymer were eluted. For this step, 3 mL of acetonitrile was placed in the syringe and then removed. Finally, the samples were transferred to a 10-mL volumetric flask and diluted with acetonitrile and were then filtered through a 0.45-μm membrane syringe filter. Then, 5 μL of this solution was injected into the HPLC-fluorescence system.

3. Results and discussion

3.1. Synthesis and characterization of the MIPs

There are some disadvantages in using MIPs as sorbents in SPE. For example, it is possible that template molecules remain trapped in the polymers, and these can leak into the samples. This would affect the quantitation of the curcuminoids [25]. The best way to overcome this problem is using a mimic of the target molecule during the design and production of the MIP [26]. MIP synthesis depends on several factors such as the monomer (styrene) to crosslinker (EGDMA) ratio, temperature, and the type and concentrations of monomer and porogenic solvent. The synthesis of a MIP for curcumin was prepared using acrylamide and MAA as monomers, EGDMA as the crosslinker, azobisisobutyronitrile (AIBN) as the initiator, and THF as the porogenic solvent [21]. In this procedure, the effects of acetonitrile and THF as porogenic solvents were investigated to identify the best porogen. The concentration of monomer to crosslinker required for the synthesis of MIPs in the presence of acetonitrile and THF is shown in Table 1. The MIPs were prepared following the description of Schirmer and Meisel [27] with slight modifications. The ability of the MIP to trap the curcuminoids was initially evaluated using HPLC-fluorescence detection. In this study, the effect of different monomers (styrene, MAA, and EMA) on the product was analyzed. Comparing MIPs and NIPs, we found that the selectivity and binding of the MIPs were higher than those of the NIPs. Additionally, the polymer prepared from styrene was found to have the best binding capacity and selectivity for curcuminoids, as shown in Fig. 1. During synthesis, the curcuminoids were used as the template molecules, styrene was the functional monomer, acetonitrile was the porogenic solvent, BPO was the initiator, and EGDMA was the crosslinker. The hydroxyl groups of curcuminoids can form hydrogen bonds with the functional groups of the polymer molecules. EGDMA was added to the mixture as a crosslinker to strengthen the polymer structure. Acetonitrile was added to increase the porosity and the surface area for analyte recognition sites. The MIP structure became rigid after 30 min of polymerization. Subsequently, the imprinted polymer sites were maintained after the removal of the template molecules.

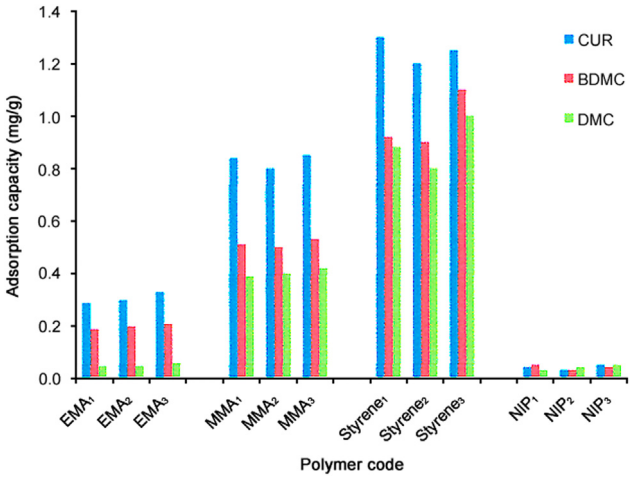

Fig. 1. The effect of monomer type on the adsorption capacity of curcuminoids (10 mg/L) on MIP and NIP(n = 3).

The adsorption of curcuminoids on MIPs (Cur_1-Cur_{12}) and NIP was tested at different contact times (up to 24 h). The binding characteristics of the curcuminoid and MIPs were investigated by comparing the results obtained using the MIPs and NIPs. The curcuminoid adsorption capacities of the MIP and NIP adsorbents were investigated. The sorption of curcuminoids by the MIPs was significantly higher than that by the NIP. The template binding for the MIPs exceeded that of the NIPs at curcuminoid concentration of 10 mg/L. After 24 h, the adsorption capacity reached equilibrium for the MIP. The effects of the type and amount of monomers and porogenic solvent were investigated based on rebinding test results. The adsorption capacity of all synthesized MIPs can be calculated using Eq. (1). The types and amount of monomer and porogenic solvent used in the preparation of the MIPs and NIP are listed in Table 1. A comparison of the adsorption capacity for MIPs and NIP indicated that the NIP provided the lowest capacity. The type of monomer which gave the highest adsorption capacity was used for further investigation. The results revealed that styrene monomer yielded a polymer with a higher adsorption capacity than those of MAA and EMA. In the same way, styrene was polymerized with 47 g of acetonitrile and 1.0238 mmol of EGDMA, and

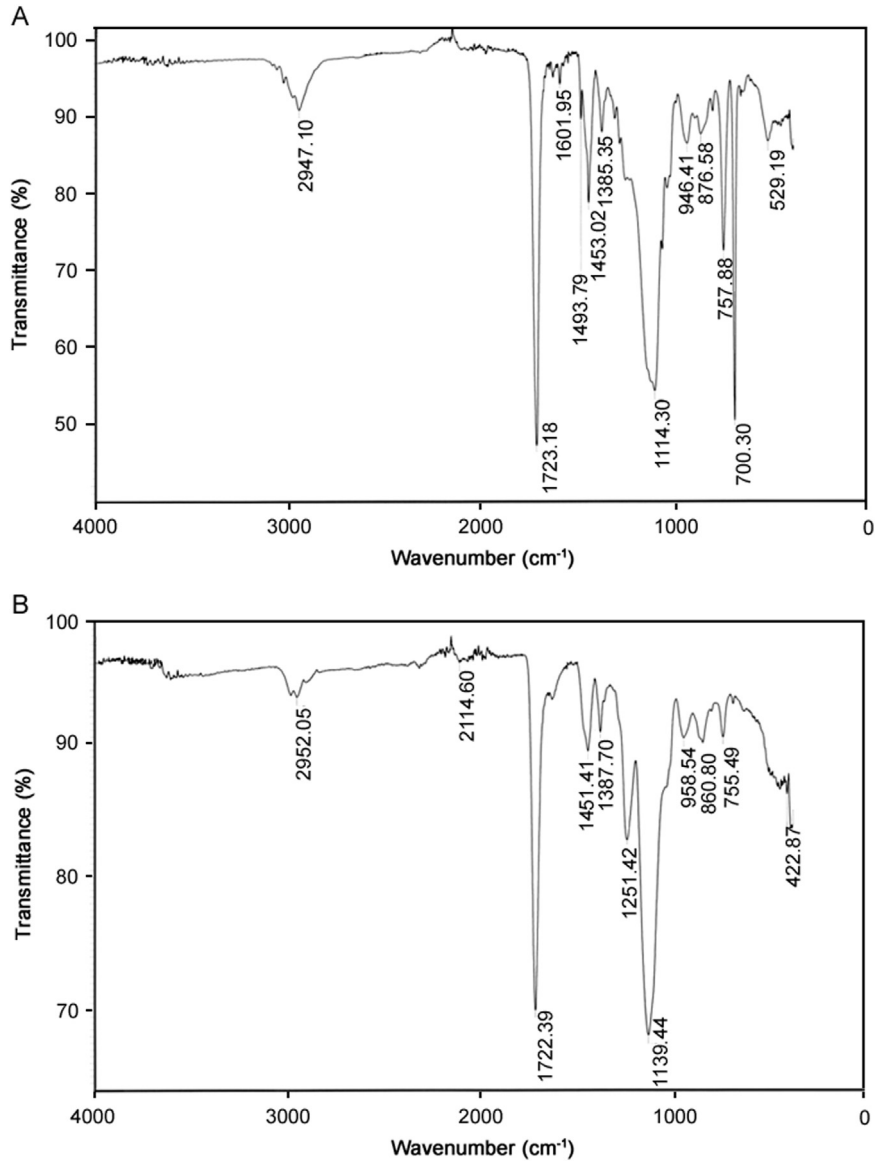

Fig. 2. Comparative FT-IR spectra of (A) Cur-MIP and (B) NIP prepared by precipitation polymerization.

this yielded a polymer with the highest binding capacity compared to the use of other monomers. The effects of the amount of template and crosslinker were investigated. The results showed that the obtained Cur_5-MIP prepared with 0.0325 mmol of curcuminoids, 1.0238 mmol of EGDMA, and 47.0 g of acetonitrile had the highest binding capacity. Acetonitrile used as the porogenic solvent increases the porosity in the polymer structure of the MIP, and, thus, the binding capacity of MIP using 47.0 g of acetonitrile was higher than that prepared with 23.5 g of acetonitrile. However, the polymer prepared with 0.5119 mmol of EGDMA and excess porogenic solvent had a low adsorption capacity because of its dilution in the pre-polymerization mixture. Thus, Cur_5-MIP was found to have the highest binding capacity and was used for reusability testing.

3.2. Structural characterization and morphology of the polymer

MIP is an extensively crosslinked polymer containing specific recognition sites with a predetermined selectivity for the analysis of the compound of interest. The imprinting process is performed by copolymerizing functional and crosslinking monomers in the presence of template molecules. The subsequent removal of the imprinting (template) molecule leaves complementary binding sites in the polymer network, allowing molecules of a similar size and shape to bind specifically. Traditional interactions between the template and monomer include hydrogen bonds, ion pairing, hydrophobic interactions, and π-π interactions [23].

3.2.1. FT-IR spectroscopy spectra of Cur-MIP and NIP

To determine the interaction sites of the template and monomer, Cur-MIP and NIP were characterized using FT-IR. The interactions between the functional monomer and template molecules were of high affinity and selectivity in Cur-MIP. In this study, the FT-IR spectrum shown in Fig. 2A was used to confirm the structure of Cur-MIP after copolymerization. The FT-IR spectrum of Cur-MIP shows a broad C–H stretching peak at 2947 cm^{-1} which could be due to the presence of methylene groups in both styrene and EGDMA. A band corresponding to the vinyl group (C–C vinyl group) of the ester group was observed in the spectrum of the MIP at 1723 cm^{-1}, and this might originate from styrene and EGDMA. Relatively weak combination C=C aromatic bands were observed between 1453 and 1385 cm^{-1}, and sharp band =C–H at 1114 cm^{-1}. Two sharp bands

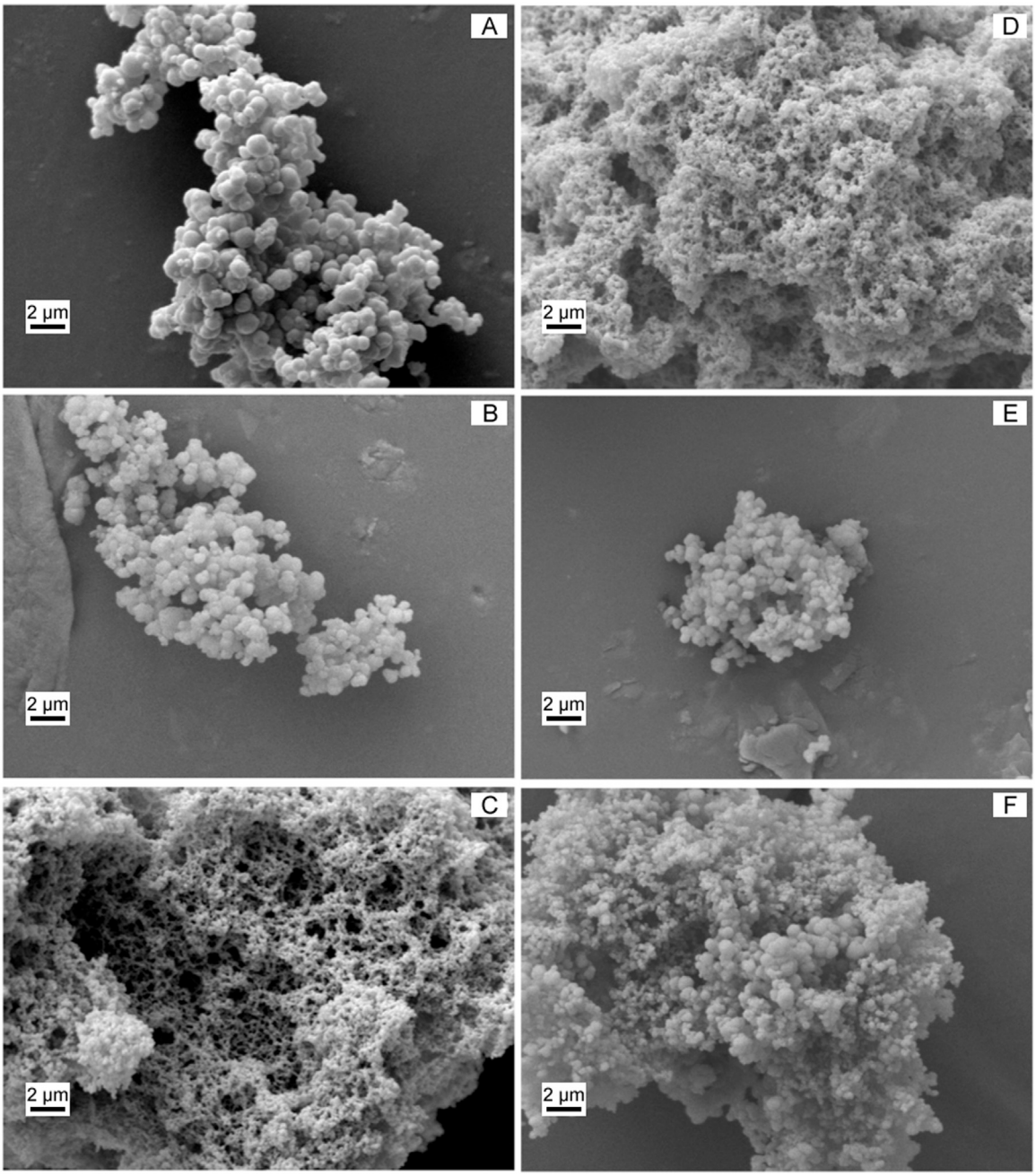

Fig. 3. Scanning electron microscopy images of the MIPs and NIPs (A = Styrene-MIP, B = Styrene-NIP, C = EMA-MIP, D = EMA-NIP, E = MAA-MIP, and F = MAA-NIP).

were observed at 757 and 700 cm^{-1} only in the MIP spectrum, relating to vibrations of the MIP. The vinyl function group (=C–H vinyl) was also seen at 757 and 700 cm^{-1}. In the FT-IR spectrum of the NIP, there were characteristic signals similar to those of Cur-MIP (Fig. 2B). However, (1) the intensity of the C–H (2952 cm^{-1}) stretch was lower than that of Cur-MIP, and (2) the intensity of the =C–H (1139 cm^{-1}) and C=C aromatic (1451 cm^{-1}) peaks were higher than those of Cur-MIP. These changes may arise because (1) the polymerization of NIP does not involve curcuminoids, so styrene yields a strong =C–H vinyl (757 and 700 cm^{-1}) vibration and (2) the curcuminoids, via hydrogen bonded interactions, are essential to the copolymerization of styrene and EGDMA. Consequently, stable imprinting cavities were formed by the ordered distribution of C=C and =C–H functional groups. This result indicates that the Cur-MIP contains cavities containing an abundance of functional groups that should result in high selectivity.

3.2.2. SEM images of both Cur-MIP and NIP

The surface morphologies and the particle sizes of both Cur-MIP and the NIP were analyzed using SEM. The results indicate that the particles obtained after the preparation of the polymers by precipitation polymerization were irregularly shaped microparticles. After the removal of the template in the Cur-MIP and NIP template, the NIP surface was smoother than that of Cur-MIP, while the Cur-MIP had rough surfaces, which can be attributed to the formation of pores during synthesis. It has been reported that the pores of Cur-MIP particles can lead to a higher surface area than that of NIP [21], and thus, Cur-MIP adsorbs analytes much better than the NIP. Fig. 3 shows the scanning electron micrographs for the three different MIPs and NIPs (Styrene, EMA, and MAA). These all have porous surfaces with pore diameter distributions ranging from 2 to 5 μm.

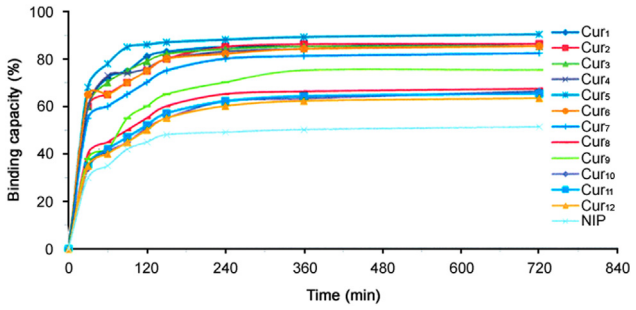

Fig. 4. The adsorption kinetic of curcuminoids on styrene-MIP (Cur_1-Cur_{12}) and NIP.

3.3. Adsorption kinetics of the Cur-MIP

The adsorption kinetics of the MIPs (Cur_1 to Cur_{12}) and NIP (see Table 1) were tested for curcuminoids at a concentration of 10 mg/L, and the samples were collected at different time intervals (0, 30, 60, 90, 120, 150, 240, 360, and 720 min) at room temperature. The curcuminoid binding capacities of the MIPs were investigated, and the binding of the target molecules was compared with the binding to NIP particles. The binding capacity can be calculated using Eq. (2). The sorption of curcuminoids by the MIPs was significantly higher than that of the respective NIP controls, suggesting that while both MIPs and NIPs can bind curcuminoids, the imprinting effect allowed the MIPs to bind significantly more curcuminoids than the corresponding NIPs. The template binding for the MIPs exceeded those of the NIPs at a curcuminoid concentration of 10 mg/L. After 120 min, it was assumed that the adsorption capacity had reached equilibrium in the MIPs. As shown in Fig. 4, the adsorption capacity of Cur_5-MIP showed the highest binding capacity, and the MIP contained the highest amount of crosslinker and porogenic solvent. The binding capacity of the NIPs was low compared with that of the MIPs, thus confirming that the NIPs do not contain complementary binding sites for curcuminoids.

3.4. Validation of the HPLC-fluorescence method

The analytical parameters of merit of the analytical method, such as linearity, limits of detection (LOD) and quantification (LOQ), repeatability, and intermediate precision, as well as the feasibility of the method through recovery studies, were evaluated using HPLC-fluorescence method.

3.4.1. The linearity of the calibration graph

The linearity of the calibration graph was evaluated by linear regression analysis based on least-squares. The linear calibration graph was obtained over a standard curcuminoid concentration range of 0.1–40 mg/L. Each concentration was tested in triplicate. Linear calibration graphs of CUR, DMC, and BDMC were obtained with correlation coefficients of 0.9966, 0.9967, and 0.9972, respectively (Fig. 5A). In this study, we carried out a comparative evaluation of the detection sensitivity of fluorescence and UV–visible detection coupled with HPLC for the determination of curcuminoid concentration. The results of this comparison study clearly indicate that the fluorescence detector had higher sensitivity than UV-visible detector (Fig. 5B).

3.4.2. LOD and LOQ

The LOD and LOQ of the method were investigated by injecting a standard solution of curcuminoids into the HPLC analytical system. The values of LOD and LOQ were calculated by analyzing the calibration curves and using Eq. (3).

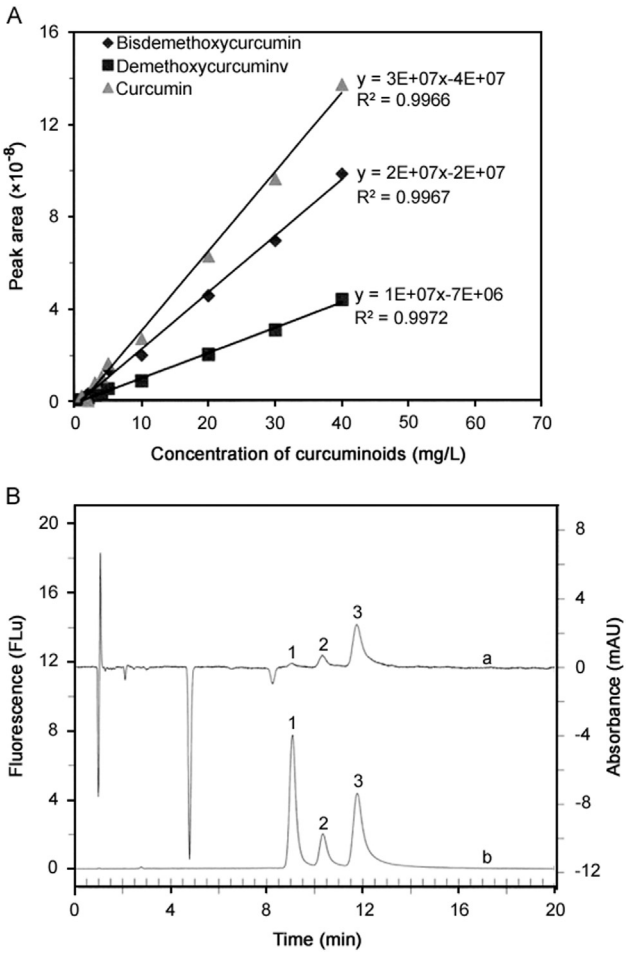

Fig. 5. (A) Calibration graph of curcuminoids standard solutions and (B) comparative HPLC chromatograms of curcuminoids at (a) 425 nm with UV light and (b) fluorescence detection (peak1 = CUR, peak2 = BDMC, and peak3 = DMC).

$$C = (K \times SD)/S \quad (3)$$

Here, SD is the standard deviation of the *y*-intercept values, and *S* is the mean slope from the three plotted calibration curves. The K values were 3 and 10 for LOD and LOQ, respectively. The LOD values of CUR, DMC, and BDMC were found to be 5.00, 20.00, and 10.00 μg/L, respectively. The LOQ values of CUR, DMC, and BDMC were found to be 16.66, 66.66, and 33.33 μg/L, respectively.

3.4.3. Precision and accuracy

Repeatability and intermediate precision were performed at three concentration levels (5, 10, and 15 mg/L). Six measurements were used for same-day repeatability assays. Intermediate reproducibility (between-day) was studied over a period of 3 days in triplicate. The repeatability was calculated and found to be in the range of 0.30%–2.82%. The intermediate reproducibility was calculated and found to be in the range of 0.63%–2.92%. The relative standard deviation (RSD)

Table 2
Precision studies for curcuminoids (mean of six determinations).

Concentration (mg/L)	Repeatability (%RSD)			Intermediate reproducibility (%RSD)		
	CUR	BDMC	DMC	CUR	BDMC	DMC
5	0.30	0.57	0.75	1.22	0.89	1.99
10	2.82	0.89	1.85	2.82	0.78	2.92
15	1.93	0.82	1.96	1.80	0.63	1.94

Table 3
Recovery of the Cur-MIP-HPLC method for CUR, BDMC and DMC analysis in various samples (mean of three determinations).

Sample	Conc. spiked (mg/L)	Conc. found (mg/L)			% Recovery (mean ± SD)		
		CUR	BDMC	DMC	CUR	BDMC	DMC
Capsule	1	1.01	0.98	0.97	101.00 ± 0.58	98.00 ± 0.90	97.00 ± 0.80
	5	4.90	4.44	4.51	98.01 ± 0.45	88.82 ± 0.70	90.29 ± 0.45
	10	9.38	9.44	9.16	93.80 ± 0.35	94.45 ± 0.69	91.66 ± 0.72
	15	13.36	13.69	13.02	89.09 ± 0.96	91.27 ± 1.00	86.81 ± 0.40
Herbal extract	1	0.98	0.98	0.95	98.00 ± 0.26	98.00 ± 0.94	95.00 ± 1.00
	5	4.94	4.38	4.37	98.95 ± 1.00	87.60 ± 1.15	87.57 ± 0.40
	10	9.46	9.07	8.78	94.66 ± 0.70	90.72 ± 1.00	87.88 ± 0.20
	15	12.90	13.20	12.57	86.00 ± 0.31	88.05 ± 0.96	83.81 ± 0.40
Urine	0.1	0.098	0.092	0.095	98.00 ± 1.00	92.00 ± 0.95	95.00 ± 0.50
	0.5	0.52	0.48	0.49	104.00 ± 0.51	96.00 ± 1.00	98.00 ± 1.00
	1.0	0.98	1.01	0.97	98.00 ± 0.20	101.00 ± 0.95	97.00 ± 0.20

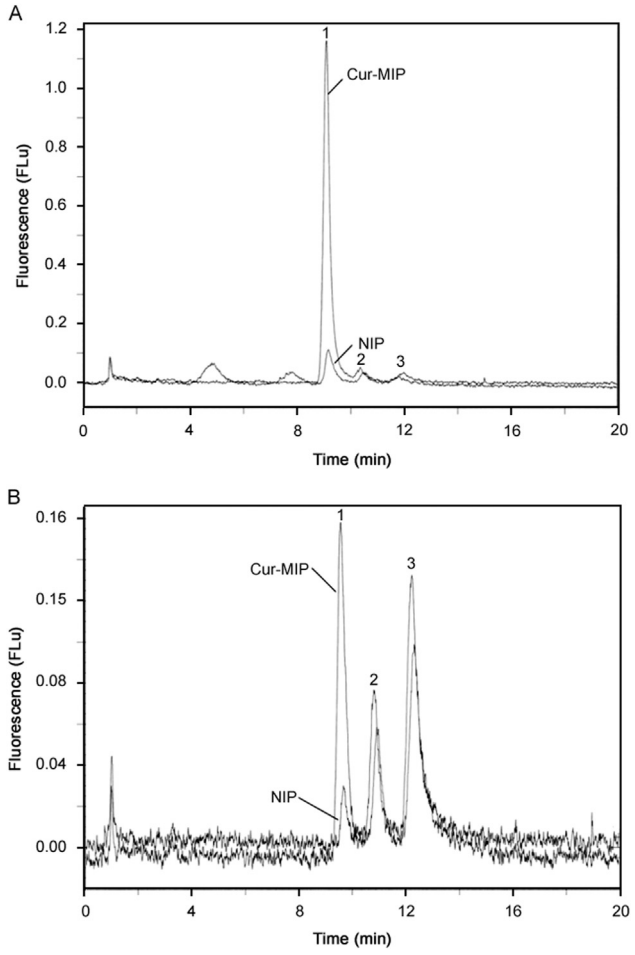

Fig. 6. Chromatograms of (A) a herbal extract sample after preliminary cleanup and the concentration of Cur-MIP and NIP and (B) a urine sample after preliminary cleanup and the concentrations of Cur-MIP and NIP (peak1 = CUR, peak2 = BDMC, and peak3 = DMC).

of the same-day, and between-day repeatability were lower than 2.82% and 2.92% respectively, indicating good precision. The results are listed in Table 2. The percentage recoveries were also determined by the standard-addition method. Curcuminoids standards (1, 5, 10, and 15 mg/L) were added and mixed with known aliquots of sample solutions. The sample solution was extracted and analyzed using the proposed method. Triplicate determinations of each concentration were conducted, along with statistical evaluation showing the standard deviation of difference values. The mean percentage recoveries of the spiked curcuminoids in turmeric capsule, herbal extract, and urine samples are presented in Table 3, with averages in the ranges of 86.81%–101.00%, 83.81%–98.95%, and 92.00%–104.00%, respectively. As listed in Table 3, recoveries in the range of 83.81%–104.00% and SD values below 2% were obtained, indicating that the proposed method is accurate for the simultaneous determination of CUR, DMC, and BDMC in real samples.

Table 4
Applications of Cur-MIP and HPLC-fluorescence method for the determination of curcuminoids in the real samples (mean of three determinations).

Sample type	Sample code	Curcuminoids content (mg/g)		
		CUR	BDMC	DMC
Turmeric (*Curcuma longa* L.)	Turmeric 1	1.17	0.46	0.65
	Turmeric 2	2.12	0.35	0.63
	Turmeric 3	10.97	0.85	1.09
Capsule	Capsule 1	19.81	0.74	3.15
	Capsule 2	35.48	0.65	3.82
	Capsule 3	21.98	0.84	3.64

3.5. The contents of curcuminoids in medicinal plant and pharmaceutical samples

The proposed HPLC-fluorescence detection method was applied for the determination of curcuminoids in turmeric extract and pharmaceutical products collected from Phitsanulok Province, Thailand. The sample solutions were prepared as described in the Experimental Section. The content of curcuminoids in each sample solution was determined using HPLC-fluorescence method. The sample solution gave well-defined peaks. The retention times for CUR, DMC, and BDMC were found to be 9.1, 10.4 and 11.9 min, respectively, as shown in Fig. 6A. There were no interference peaks present in either sample solution. The average contents of curcuminoids of all sample solutions are shown in Table 4.

3.6. The determination of curcuminoids in urine samples

The HPLC method used for the analysis of curcuminoids in the urine sample was originally developed for the analytical assay of curcuminoids in an aqueous matrix without the addition of an internal standard [28,29]. The extraction efficiency for curcumin

has not been described for this method. Therefore, it is possible that method sensitivity may be a factor in the non-detection of curcumin in the urine of patients from a reported phase I study by Cheng et al. [28]. In the present study, the urine sample was spiked with the curcuminoid standard solution. The proposed method was applied to determine the concentration of curcuminoids in the spiked urine sample. The sample solution was extracted using the MIP extraction steps and the concentrations were determined using HPLC-fluorescence detection. The chromatograms of sample solutions after loading Cur-MIP and NIP are shown in Fig. 6B.

4. Conclusion

This work demonstrates the usefulness of templates for the preparation of MIPs selective to CUR, BDMC, and DMC. A mixture of curcuminoids was the best choice for the generation of binding cavities with the right shape and functionality to achieve the efficient recognition of the target curcuminoids in turmeric samples. This approach avoids the problems typically associated with copolymerization in the presence of curcuminoids during the preparation of MIPs. A Cur-MIP was synthesized from 0.0237 mmol of styrene, 47.0 g of acetonitrile, 1.0238 mmol of EGDMA, 0.0325 mmol of curcuminoids, and 0.2480 mmol of BPO. The sample solution was separated by Cur-MIP using SPE. Subsequently, the sample solution was analyzed on Brownlee analytical C_{18} column (150 mm × 6 mm, 5 μm) using an isocratic elution consisting of acetonitrile and 0.1% TCA (40:60, v/v). The flow rate was maintained at 1.5 mL/min. The fluorescence detector was set to monitor at $\lambda_{ex} = 426$ nm and $\lambda_{em} = 539$ nm. The quantification limit values were found to be 16.66, 66.66, and 33.33 μg/L for curcumin, demethoxycurcumin, and bisdemethoxycurcumin, respectively. The proposed method was validated and found to have good recoveries and precision. The proposed Cur-MIP was successfully applied to the selective enrichment of curcuminoids extracted from medicinal plant and pharmaceutical products and biological samples, and the curcuminoid content was directly determined by HPLC with fluorescence detection. The present method is simple, selective and accurate, and provides a promising alternative to traditional SPE sorbents for the extraction and determination of curcuminoids.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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