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Direct extraction of specific pharmacophoric flavonoids from gingko leaves using a molecularly imprinted polymer for quercetin

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Abstract

A new straightforward approach to extract active components from Chinese traditional herb was reported by using a molecularly imprinted polymer (MIP) as the sorbent material. The MIP was prepared using quercetin, a typical active compound of the flavonoid family in gingko leaves as the template. Acrylamide was used as the functional monomer and two polar solvent tetrahydrofuran was used as the porogen. Afterwards, the polymer was evaluated by chromatographic analysis. It exhibited high selectivity for quercetin and good affinity to its structural analogues. Specific binding amount of quercetin on the MIP in the solvent methanol was 12 μg/g polymer by the cartridge test. The MIP cartridge could directly trap a specific class of compounds including quercetin and kaempferol from the hydrolyzate of gingko leaves. The result demonstrated the possibility of direct extraction of certain pharmacophoric constituents from herb by MIP technology. The compounds extracted were confirmed by time-of-flight mass spectrometry. © 2001 Published by Elsevier Science B.V.

Keywords: Molecular imprinting; Sorbents; Solid-phase extraction; Plant materials; Flavonoids; Quercetin; Kaempferol; Acrylamide

1. Introduction

Molecular imprinting is an emerging technique for the preparing polymeric materials possessing highly selective and affinitive properties. The method involves complexation in solution of a target compound (template) with functional monomers, through either covalent or non-covalent bonds followed by a polymerization reaction with an excess of cross-linkers. Removal of the template molecule by extracting with solvents leaves behind specific recognition sites that are complementary to the template in terms of its shape, size and functionality in the polymer network. Molecularly imprinted polymers (MIPs) show higher selectivities and affinities in rebinding the template and its analogues than other structurally unrelated compounds [1,2].

MIPs have been used in areas in which selective recognition of particular molecules is necessary or desirable including separations (e.g., chiral separations) [3–5], immunoassay [6–8], sensors [9] and

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catalysis/artificial enzymes [10,11]. Recently MIPs have attracted considerable attention as solid-phase extraction (SPE) sorbents for the clean up and preconcentration of samples before determining drugs in complex biological fluids [12–17], nicotine in chewing gum and tobacco [18,19], and triazine herbicides in beef liver [20], and water [21,22]. However, the use of MIPs as separation materials for extracting certain active components directly from herb has never been reported as far as we know.

Generally, extraction or separation of active components from herb is tedious and inefficient resulting from poor affinity and selectivity of conventional separation materials (e.g., normal-phase, reversed-phase, ion-exchange types). Much research has been directed toward the variation of the sorption materials in order to achieve the selective interaction with the target molecule [23]. The immunoaffinity type adsorbents in which antibodies and biomolecules are immobilized are generally expensive, not straightforward to prepare, and unstable in most media, thus limiting their use in the herb matrix.

It was expected that MIPs could be potentially used as separation materials in extracting certain active components directly from herb based on their such features as the shape, size and functionality selectivity, strong affinity on rebinding target compounds, the significantly low cost for the preparation, and the workability in organic solvents [24,25].

In this study, a selective and affinitive MIP was prepared for quercetin using a non-covalent imprinting approach in the polar solvent tetrahydrofuran (THF). Characteristics of this polymer were evaluated by HPLC. Also its application for extracting compounds of the flavonoids from gingko leaves was investigated. Quercetin was chosen as a representative target compound because it is a typical member of the large family of flavonoids, which can be used for the treatment of hypertension, higher blood-fat, etc., and is commonly found in herbs, for instance, in gingko leaves. The work described in this paper was undertaken in order to show the feasibility of direct extraction of specific pharmacophoric flavonoids from gingko leaves by the MIP. Our approach expected to offer a quick and simple separation technique for extracting certain active components from herb.

2. Experimental

2.1. Reagents

Acrylamide, ethylene glycol dimethacrylate (EDMA), azobis(isobutyronitrile) (AIBN), quercetin, chrysin, morin, and narengenin were bought from Acros Organics (Geel, Belgium). OHP (2-oxo-4,5,-dihydroxy-3-methylpyrane) and GHF (7-*o*-glucosyl-3′-hydroxy-4′-methoxyisoflavone) were kindly donated by School of Pharmacy, Peking University. Gallic acid and rutin were products of the National Medicinal Company, China. Before use, the EDMA was distilled under vacuum after being extracted with 10% sodium hydroxide brine and dried over anhydrous magnesium sulfate. AIBN was recrystallized from methanol, and THF (analytical-reagent grade) was dried by sodium and distilled. Methanol and acetonitrile were HPLC grade, glacial acetic acid was analytical grade, and the other chemicals were used as obtained. The water was demineralized and purified by a Millipore system.

Phosphoric acid solution was prepared by dissolving 8.0 g H_3PO_4 (analytical grade) in 2.5 l water and then adjusted to pH 2.85.

2.2. Equipment

An HP1100 HPLC system with a quaternary pump, a variable-wavelength detector, an on-line vacuum degasser and a 20-μl manual injector (Hewlett-Packard, Palo Alto, CA, USA). A Mariner time-of-flight (TOF) MS system with an electrospray ionization (ESI) interface (PE PerSeptive Biosystems, Framingham, MA, USA) was used. The ion polarity was negative. The nebulizer gas and curtain gas were both nitrogen, at flow-rates of 0.5 and 1.5 ml min^{-1}, respectively. The nozzle and quadrupole were both heated to 140°C. The spray tip and nozzle potentials were 5000 and 100 V separately. The acquisition speed was 3 s per spectrum.

2.3. Preparation of the MIP

The monomer acrylamide (4 mmol), the imprint molecule quercetin (0.8 mmol), and the porogen THF (9 ml) were placed into 18×180 mm borosili-

cate glass test tubes, the crosslinker EDMA (40 mmol) was then added, followed by the reaction initiator AIBN (22.0 mg). After shaking for homogeneity, the mixture was sparged with nitrogen for 20 min, and then sealed under vacuum. The polymerization was carried out in a water bath at 60°C. After 24 h, the product polymer was ground and passed through a 30-μm sieve, fine particles were removed by repeated flotation in acetone and then dried under vacuum. The polymer was packed into stainless steel columns (150×4.6 mm I.D.), and washed on-line in the HPLC system one by one with methanol–acetic acid (9:1, v/v), methanol, methanol–triethylamine (9:1, v/v), and finally methanol until a stable baseline was obtained. Some columns were used in the HPLC test; the others were emptied out to prepare the polymer for the cartridge use. As a control, non-imprinted blank polymer (BP) in the absence of the template was also prepared and treated in the identical manner. In addition, the BP was studied in parallel in the same way as the MIP in the following sections.

2.4. Chromatographic study

HPLC evaluation of the MIP column was performed isocratically at room temperature. The flow-rate was 1.0 ml min^{-1}; the sample volume and concentration injected were 20 μl and 0.1 mmol l^{-1}, respectively. Detection was carried out using UV absorption at 254 nm. Acetone was used as a void marker. Each sample was injected independently. The capacity factor k' was calculated using the equation, $k' = (t_R - t_0)/t_0$, where t_R was the retention time of a sample and t_0 was the time to elute acetone. The imprinting effect (IF) was defined by the equation IF = k'(MIP)/k' (BP), where k' (MIP) was the capacity factor on the molecularly imprinted polymer, k' (BP) is the capacity factor on the blank polymer. The relative retention value (α) was calculated as the ratio of the capacity factor of the template to the capacity factor of the examined molecules.

2.5. Cartridge capacity test

The 0.3517-g amount of dry polymer was packed into a 6.0-ml polypropylene cartridge. The cartridge was fitted with a syringe connector. Prior to applying the sample, the polymer was pre-equilibrated with 10 ml methanol. Aliquots (1 ml) of 0.8 μg ml^{-1} quercetin in methanol were applied gradually to the cartridges until release was detected. And then the cartridge was washed with two aliquots (1 ml) of methanol, followed by eluting with three aliquots (1 ml) of methanol–acetic acid (9:1, v/v). The effluent liquids from each application, and wash step were collected and analyzed directly, while the fractions at the elution step needed to be evaporated to dryness under vacuum to remove acetic acid and then reconstituted in methanol before analysis. Sample analysis was conducted by a HPLC system with a guard column (DIKMA) and an analysis column (Hewlett-Packard. Zorbax Eclipse XDB-C_8 150×4.6 mm I.D.). The mobile phase [26,27] consisted of solvent A (methanol), and solvent B (the prepared phosphoric acid solution) with the following gradient (%A): 0 min, 38%; 30 min, 55%; 31 min (washing), 100%; 41 min (washing), 100%; and 42 min (equilibration), 38%. The sample size injected was 20 μl. The flow-rate was 1 ml min^{-1}. Detection was at 365 nm. The column temperature was ambient.

2.6. Extraction test

Cartridge preparation, fractions collection and HPLC analysis were the same as described in Section 2.5. While in this test, five samples (200 μl) of the hydrolyzate of gingko leaves were loaded onto the cartridge, followed by washing with 15 samples (200 μl) of methanol. And the cartridge was completely eluted using 3 ml methanol–acetic acid (9:1, v/v).

The hydrolyzate of gingko leaves was prepared as reported before [26,27]. One gram of dried and pulverized gingko leaves was thoroughly extracted with soxhlet using methanol, reduced to dryness by a rotary evaporator under vacuum, and the residue dissolved in 30 ml methanol. After 5 ml 25% hydrochloric acid was added, it was refluxed for 1 h in a 80°C water bath. The obtained solution was repeatedly reduced under vacuum until no hydrochloride was detected by pH test paper. Finally the volume of the solution was fixed to 150 ml with

methanol. The solution was filtered through a Bond-Elut C_{18} cartridge before use. The contents of quercetin in the hydrolyzate were determined with the above HPLC gradient using quercetin as an external reference.

3. Results and discussion

3.1. Polymer preparations

In most of the present studies, the imprinted particles were obtained from bulk polymerization by non-covalent approach. Up to now, successful imprints have been made with affinity to various classes of compounds, for instance, amino acids derivatives [4], sugars and sugar derivatives [28,29], and drugs such as bronchodilators and tranquilizers [30] by this method. However, it is generally believed that compounds of solubility in polar solvents are not desirable used as templates because polar solvents can disturb the non-covalent cohesions between the templates and functional monomers. Quercetin is a strong polar molecule possessing no hydrophobic functional groups, which can be dissolved in THF at the usual imprinting concentration. Acrylamide was chosen as the functional monomer because it is favorable for methacrylic acid associating with templates by hydrogen-bonding interaction in polar environment [31]. Our successful imprinting of quercetin owed to the cohesions between quercetin and acrylamide strong enough for the decomposing action made by THF. Consequently compounds of solubility in polar solvents still could be imprinted by non-covalent method in polar solvents as long as the negative action caused by polar solvents was not so strong as to be complexation impeding. Excess EDMA was used in order to enhance the imprinting effect. And prior to polymerization, the mixture was thoroughly deoxidized in case of free-radical reaction quenching [32].

3.2. Chromatographic study

3.2.1. Eluent study

MIPs have so far been characterized mainly by HPLC. Thus, we decided to examine the polymer performance in the HPLC system in order to verify the imprinting effect. Table 1 summarizes the investigation result in different eluents. It is known that quercetin can form hydrogen-bonding interaction with acrylamide. First acetonitrile was used as the mobile phase, which is commonly used to perform chromatographic tests with hydrogen-bonding based molecular imprints [33]. As the solvent acetonitrile was used, quercetin could not be eluted in 103 min from the MIP column, exhibiting the longest retention times. With increasing water content, the capacity factor was significantly decreased. As the water content was 7% (v/v) in the eluent, the MIP almost lost its imprinting effect. This showed that hydrogen bonding was the dominant interaction in the binding. When the mobile phase was changed to methanol, capacity factor and the imprinting effect (IF) value decreased evidently because the polar and protic solvent methanol more strongly disturbed quercetin's hydrogen bonding interaction with amido groups on the polymer. The IF was still quite considerable in methanol, but the chromatographic peak appeared broad. When the more polar and protic solvent, methanol–acetic acid (9:1, v/v), was used, a symmetrical chromatographic peak shape was obtained but the imprinting effect disappeared. All of this suggested that quercetin's retention on the

Table 1
Chromatographic performance of quercetin in different mobile phases

Mobile phase		Acetonitrile	(1–4%) Water in acetonitrile	7% Water in acetonitrile	Methanol	10% Acetic acid in methanol
K	BP	10	<4	3	2.0	1.5
	MIP	>39	>15	3	7.14	1.5
IF		>3.9	>3.8	1	3.57	1

The test was conducted with a column (150×4.6 mm I.D.) packed with a quercetin-imprinted polymer (MIP) or a nonimprinted blank polymer (BP). The sample volume and concentration injected were 20 μl and 0.1 mmol l^{-1}, respectively. The flow-rate was 1 ml min^{-1}. Detection was carried out using UV absorption at 254 nm. Acetone was used as a void marker.

MIP was mainly based on a hydrogen-bonding interaction mechanism, and evident imprinting effect was formed during the imprinting process on the MIP. As is obvious, column efficiency is insufficient and the column is not suited for direct separations and quantifications. Column efficiency depends on a number of variables such as particle size, morphology, packing homogeneity, and the pore size of the stationary phase. Polymer particles prepared by bulk polymerization method are usually not favorable for column use because their irregular sizes and shapes. The preparation of uniformly sized stationary phase can improve column efficiency remarkably [34]. Another significant cause of poor column efficiency is the possibility of different types of binding sites within this polymer. The initial pre-organization process may give rise to the formation of different modes of interaction between quercetin molecules and functional groups on the monomer molecules. This will result in the creation of different recognition sites in the polymer. The variable binding energies or sorption kinetics of these sites would produce retention of quercetin to different extent and hence cause an overall appearance of peak broadening. A report by Kempe and Mosbach [35] verified the use of a different cross linker, trimethylolpropane trimethacrylate (TRIM) that would be superior in terms of load capacity, selectivity, and resolving capability of the resulting MIP stationary phases for liquid chromatography. Also preparation of the polymer at a lower temperature, which is favorable to stable the interaction between the template and the functional monomer, can obtain a MIP with better recognizing properties.

3.2.2. Selectivity of the quercetin-imprinted polymer

The selectivity test was carried out using the samples, which were comparable to the compounds existing in the hydrolyzate of gingko leaves. And methanol, the common solvent in extraction of gingko leaves, was used as the mobile phase. The chemical structures of quercetin and the test compounds are shown in Fig. 1. The relative retention values (α) and the imprinting efficiency value (IF) are listed in Table 2.

The quercetin-imprinted polymer exhibited stronger affinity to the flavonoids than to the structurally unrelated compounds, and most of the flavonoids were inclined to retain on the MIP column than on the BP column. Moreover, the MIP showed the highest selectivity for quercetin among the tested compounds. Consequently it appeared that selectivity of the MIP for quercetin was induced during the imprinting process.

The selectivity test results may also give aspects of the molecular recognition mechanism. This is reflected in the relative retention values (α). The high α values showed pronounced nonspecific binding of gallic acid, rutin, OHP and GHF. The size and shape recognition by the polymer network possibly accounts for the poor retention of the four compounds. The smaller spatial structure of gallic acid or OHP leaves the two molecules poorly matching the microcavitiy induced by quercetin. While steric hindrance makes rutin or GHF difficult to be accommodated by the quercetin imprint. Morin resembles quercetin closely in structure, but it did not exhibit high ''cross-reactivity'' on the MIP (Table 2) possibly resulting from intramolecular hydrogen bonding formed in morin at the test conditions. However, at lower sample loads, the retention difference between morin and quercetin was smaller on the MIP. The extent of intramolecular hydrogen-bonding existed in morin under the test conditions is not known. Owing to the fact that the MIP showed good selectivity and affinity for quercetin, it was used in the following investigation.

3.2.3. Capacity of the cartridges

Considerations in this test were for the sake of using the cartridge on the following extraction study. The concentration of the quercetin solution loaded was higher than that for the usual SPE investigation to match the extraction test, and the solvent used in the quercetin solution was methanol. As we know, the choice of the type of solvent is very critical for the affinitive property and thus the capacity of the polymer. A polar solvent disturbs the specific noncovalent interaction between the analysts and the polymer matrix, so that decreases the capacity. The result in the chromatographic study demonstrated the MIP exhibited less affinity for quercetin in the eluent methanol than in the eluent acetonitrile. However, the polar and protic solvent methanol was selected to evaluate the capacity of the cartridge because metha-

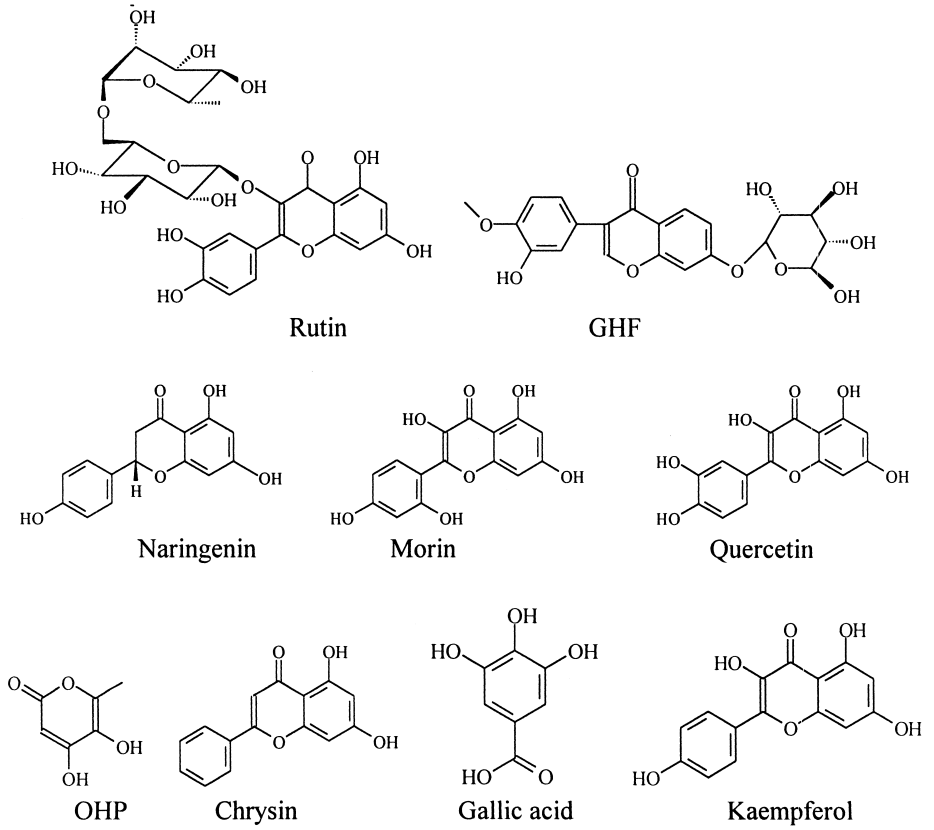

Fig. 1. Structure of the compounds discussed.

nol could sufficiently dissolve the various components in the hydrolyzate of gingko leaves.

From the results listed in Table 3, it can be seen that though the initial amount loaded on the BP cartridge was lower than that on the MIP cartridge, part of the loaded molecules were still released from the BP cartridge at the applying step because of nonspecific adsorption of quercetin on the BP. Moreover, the amount of quercetin removed from the BP cartridge at the washing step was more than that

Table 2
Chromatographic parameters for quercetin and other compounds

		Quercetin	Chrysin	Naringenin	Morin	Rutin	OHP	GHF	Gallic acid
K'	MIP	7.14	2.24	2.02	2.10	0.49	0.10	0.32	0.29
	BP	2.00	2.77	1.18	1.05	0.63	0.10	0.40	0.63
α	MIP	1	3.19	3.53	3.40	14.6	71.4	22.0	24.6
	BP	1	0.72	1.69	1.90	3.17	20.0	5.0	3.17
IF		3.57	0.81	1.71	2.0	0.78	1	0.8	0.46

The test was conducted with a column (150×4.6 mm I.D.) packed with a quercetin-imprinted polymer (MIP) or a nonimprinted blank polymer (BP). Each sample was injected independently. The sample volume and concentration injected were 20 μl and 0.1 mmol l^{-1}, respectively. Chromatographic conditions: mobile phase, methanol; flow-rate, 1 ml min^{-1}. UV detection, 254 nm.The relative retention value (α) was calculated as the ratio of the capacity factor of the template to the capacity factor of the examined molecules. The imprinting effect (IF) was defined by the equation IF=k' (MIP)/k' (BP), where k' (MIP) was the capacity factor on the molecularly imprinted polymer, k' (BP) is the capacity factor on the blank polymer.

Table 3
Sorbed and released amount of quercetin in the capacity test

Cartridge	Amount (μg) of quercetin	
	BP	MIP
Loaded	2.4	6.0
Sorbed	2.0	5.8
Washed	0.6	0.2
Extracted	1.2	5.4

The quercetin in methanol 0.8 μg ml^{-1} was applied gradually to the cartridge packed with polymer particles until release was detected. And then the cartridge was washed with two aliquots (1 ml) of methanol, followed by eluting with three aliquots (1 ml) of methanol–acetic acid (9:1, v/v). Fractions from application, wash and elution steps were collected and analyzed by an analysis column (Zobax Eclipse XDB-C_8, 150×4.6 mm I.D.). Detailed chromatographic conditions are described in Section 2.

from the MIP cartridge. Table 3 also shows that the final amount recovered for the MIP cartridge was 5.4 μg and the recovery yield was 90%. Further, the final specific amount on MIP was 4.2 μg, which was calculated by the deduction of the nonspecific adsorption amount 1.2 μg on the BP from the 5.4 μg amount on the MIP. Therefore it could be concluded that a higher capacity could be achieved by imprinting method. The capacity value of the prepared MIP was acceptable for trapping certain trace amount active compounds from herb. Moreover, capacity of the MIP can be further increased by optimizing the polymerization step.

3.2.4. Extraction test

Firstly, the content of quercetin in the prepared hydrolyzate of gingko leaves was calculated with quercetin as an external standard, and it was 2.7 μg ml^{-1}. Because deciduous leaves were used in this study, the content of quercetin was somewhat lower. Minor peaks of isorhamnetin and other flavonoids in the hydrolyzate were not discussed in this paper.

At the beginning, total amount of 1 ml or 1.5 ml hydrolyzate was tried applying to the MIP cartridge, respectively. However, when 1.5 ml was applied onto the BP cartridge, release of some components was detected (not shown in detail) because of the smaller capacity of the BP cartridge. To contrast the two cartridges, 1 ml was adopted at the applying step to cause all the compounds in the hydrolyzate of gingko leaves be retained on both the BP cartridge

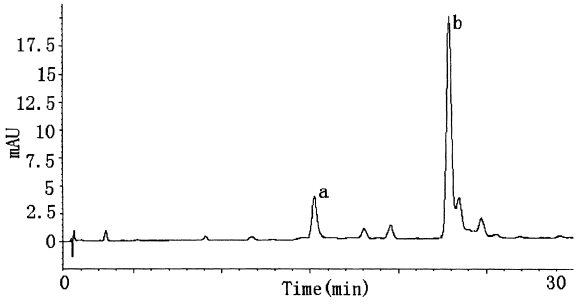

Fig. 2. The chromatogram of the hydrolyzate of gingko leaves.

and the MIP cartridge. The effluent, the washing-out solution and the final eluent from either the MIP or the BP cartridge at every applying, washing and eluting step were all analyzed by HPLC, and the major analysis results at this extraction test are shown in the chromatograms (Figs. 2–5). In these chromatograms, peak (a) was identified as quercetin; peak (b) was identified as kaempferol. It could be observed from Fig. 2 that many matrix components existed in the initial hydrolyzate. At the beginning of the washing, no visible difference exhibited between the washing-out solution from the MIP and that from the BP by chromatographic analysis. This indicated nonspecific binding took some weight in compound adsorption on the polymers. However, when the cartridges were washed for the tenth time (2 ml, cumulative amount), an evident difference was observed. Quercetin was washed out from the BP while it was not from the MIP because of the MIP's stronger affinity to quercetin compared with the BP (see Figs. 3 and 4, and Table 4). For the purpose of obtaining the fraction of better purity, washing was not stopped until almost no minor matrix peaks could be detected in the HPLC analysis. It can be seen

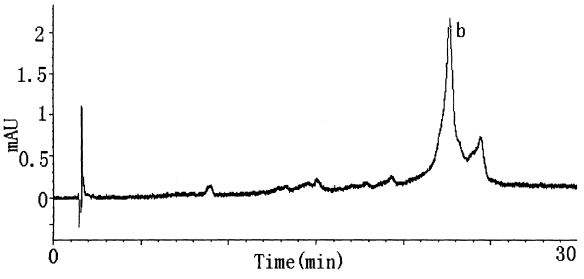

Fig. 3. The chromatogram of the tenth washing-out solution from the MIP cartridge.

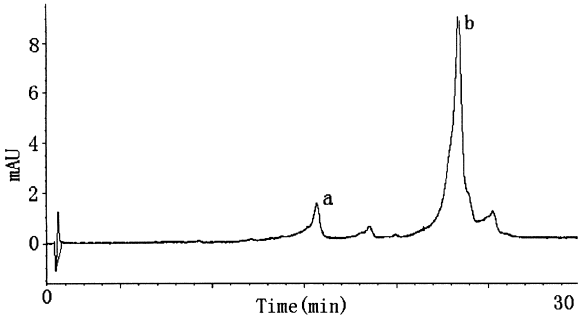

Fig. 4. The chromatogram of the tenth washing-out solution from the BP cartridge.

Table 4
Peak areas (arbitrary units), contents of quercetin for the HPLC analysis result in the extraction test

		Area, peak a	Content (μg/ml), quercetin
The initial hydrolyzate of gingko leaves		96.4	2.7
The tenth washing-out solution from the cartridges	MIP	0.0	0.0
	BP	64.0	1.7
The final eluents from the cartridges	MIP	85.0	2.4
	BP	0.0	0.0

Five samples (200 μl) of the hydrolyzate of gingko leaves were applied onto a cartridge packed with a quercetin-imprinted polymer (MIP) or a nonimprinted blank polymer (BP), followed by washing with 15 samples (200 μl) of methanol. And finally the cartridge was completely eluted using 3 ml methanol–acetic acid (9:1, v/v). Fractions from each application, wash and elution step were collected and analyzed by an analysis column (Zobax Eclipse XDB-C_8, 150×4.6 mm I.D.). The results listed in this table correspond with the chromatograms from Figs. 3–6.

from Figs. 5 and 6 that the chromatograms of the eluents were simplified as a result of the removal of matrix compounds by washing with methanol. Furthermore, after the MIP cartridge process, not only

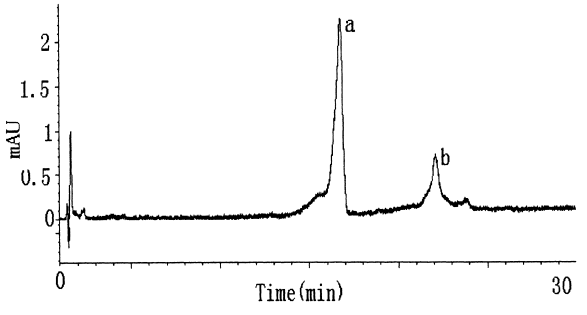

Fig. 5. The chromatogram of the eluent from the MIP cartridge.

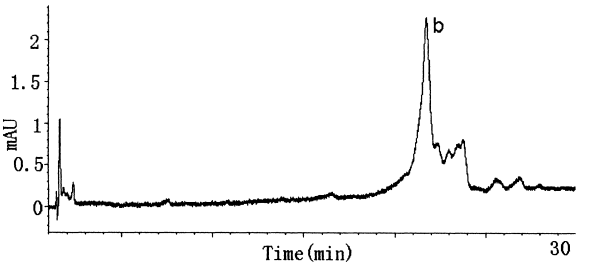

Fig. 6. The chromatogram of the eluent from the BP cartridge.

component of the template quercetin but also its analogue kaempferol was purified from the hydrolyzate of gingko leaves, and recovery yield of quercetin was satisfactory (Fig. 5 and Table 4). In contrast, after the BP process, except for kaempferol, whose content was the largest in the initial hydrolyzate according to peak areas, almost no quercetin was detected (Fig. 6 and Table 4). In view of the above capacity test (Table 3), total quercetin in the hydrolyzate could be retained by specific binding at the 1 ml loading amount. So after the MIP process, most of the quercetin was recovered. This demonstrated in the complex system of the crude herb extract was the MIP still able to exhibit the strongest affinity to its template. Though kaempferol was finally trapped by both the BP and the MIP cartridge, its retention mechanism on the two polymers might be different considering its close similarity with quercetin in structure (Fig. 1). Kaempferol could exhibit stronger affinity to the quercetin imprint than to the BP because its shape, size and functionalities almost could match the microcavity formed by quercetin. The lower yield of kaempferol after the MIP process was possibly due to the insufficient capacity of the cartridge or was limited by our extraction procedure. However, we could not further study the performance of kaempferol on the anti-quercetin MIP because it was not available commercially. Anyway, it was feasible for the simultaneous extraction of quercetin and its close structural analogue kaempferol from the hydrolyzate of gingko leaves by the MIP. Therefore directly extracting certain pharmacophoric components from herb by the MIP technology was promising in view of the sructure–active correlation principal. This is the main direction explored in our present research program.

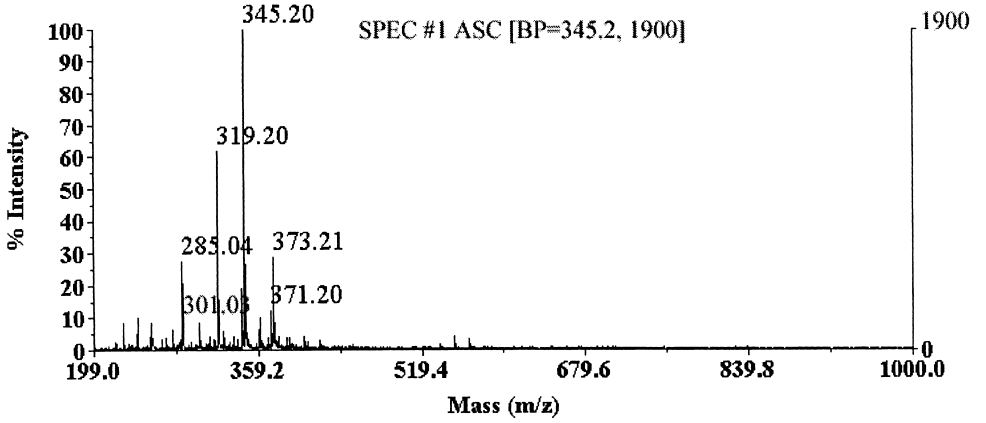

Fig. 7. The MS spectra of the initial hydrolyzate of gingko leaves.

3.2.5. Elucidation of the eluents by ESI-TOF-MS

Mass spectrometry is the preferred technique for confirmation of suspected compounds due to its inherent specificity and sensitivity. TOF-MS possesses high response and resolution properties and is capable of detecting trace components trapped by the MIP cartridge. The initial hydrolyzate and the final eluents at the extraction test were further elucidated at identical conditions by TOF-MS without being repurified by other methods. And the analysis results are listed in Figs. 7–9. In the MS spectra, main peaks were identified as follows: quercetin, m/z 301; kaempferol, m/z 285. In the spectra of the initial hydrolyzate (Fig. 7), the peaks m/z 301 and m/z 285 were not prominent among the peaks because there were various matrix compounds in the initial herb extract. And the peak m/z 301 was lower than the peak m/z 285. This was probably because the content of kaempferol in the initial hydrolyzate was higher than that of quercetin. It could be observed, the peak m/z 301 in Fig. 8 or the peak m/z 285 in Fig. 9 was projected out since quercetin or kaempferfol became the main component in the corresponding eluent after matrix compounds were eliminated by washing the cartridges. Furthermore, in the MS spectra (Fig. 8) of the eluent from the MIP cartridge, the peak m/z 285 indeed existed. The above MS result further verified the conclusion derived from

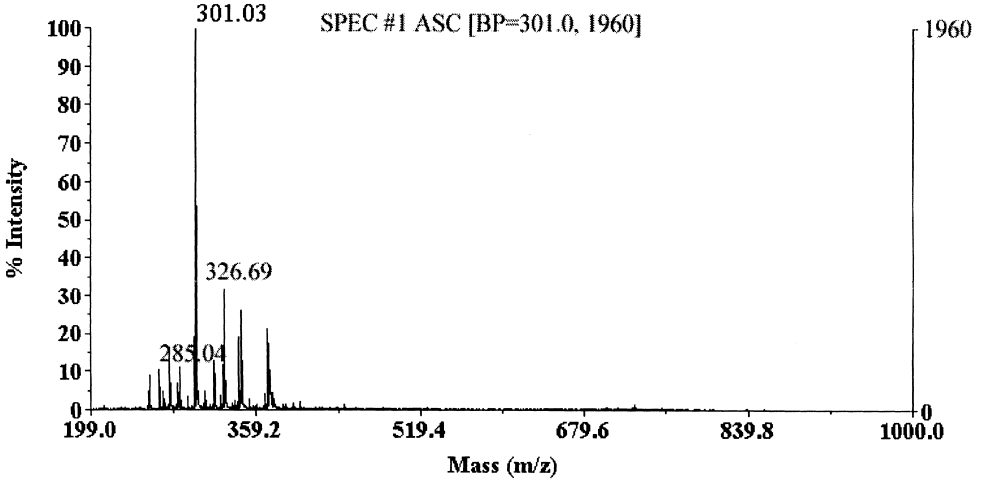

Fig. 8. The MS spectra of the eluent from the MIP cartridge.

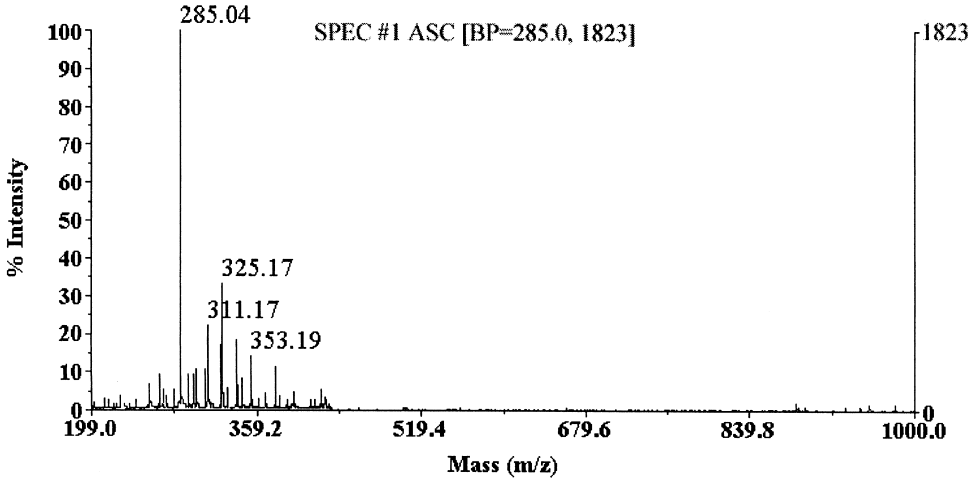

Fig. 9. The MS spectra of the eluent from the BP cartridge.

the HPLC analysis, that quercetin and kaemperfol could be simultaneously grasped from the herb matrix by the MIP cartridge (Fig. 5), while by the BP only kaemperfol could be obtained (Fig. 6). What is more, from the MS spectra of Figs. 8 and 9, it could also be seen that the matrix peaks in the eluent from the MIP cartridge were less than those from the BP eluent; therefore the purity of the eluent from the MIP was better. This was reasonable considering the retention mechanism on the two cartridges. The MIP preferred to bind the template and its structurally close analogues, but exhibited nonspecific and weak adsorption for the other components. So with the gradual wash with methanol, the nonspecific components could be easily removed while quercetin and kaemperfol were still retained by the MIP, and consequently the components of quercetin and kaemperfol were purified from the complex mixture. In contrast, the extraction on the BP was based on a nonspecific adsorption mechanism. Both the matrix compounds as well as the target compounds could be simultaneously washed out by methanol, and probably only the trace components in the initial hydrolyzate could be eliminated with most components still left in the final eluent. The results derived from TOF-MS were consistent with the HPLC analysis in the extraction test and further validated directly trapping active compounds of certain structural features by MIP technology was operable.

4. Conclusions

This study has shown that MIPs can provide efficient extraction of certain active components from herb matrix. A specific class of pharmacophoric compounds, quercetin and kaempferol, were directly trapped from the hydrolyzate of gingko leaves by the MIP process. In addition, the MIP exhibited the highest affinity to the template, and showed good workability in the herb matrix. The MIP did retain a range of molecules structurally similar and dissimilar to the template molecule. However, such matrix compounds could be removed by carefully washing with solvents, and close *structural* analogues and the template could be purified simultaneously in this way. This is promising for the further use of MIPs in grasping compounds with certain pharmacophoric features from herb in view of the structure–active correlation principle.

The molecular imprinting technique is still in a developing stage, and the performance of the MIP may be further improved, e.g., by using different cross linkers, novel functional monomers or by the covalent approach that provide stronger specific interactions with target compounds. Further studies to explore the specificity and affinity of this imprint for structurally similar molecules and the optimized conditions trapping structurally similar molecules from herb are under way.

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