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Journal of Chromatography B, 788 (2003) 233–242
JOURNAL OF CHROMATOGRAPHY B
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Selective extraction of functional components derived from herb in plasma by using a molecularly imprinted polymer based on 2,2-bis(hydroxymethyl)butanol trimethacrylate

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Received 1 July 2002; received in revised form 27 September 2002; accepted 7 October 2002

Abstract

To make molecularly imprinted polymer (MIP) solid-phase extraction (SPE) capable of direct clean-up of aqueous biological samples, an anti-quercetin MIP with evident hydrophobic matrix was synthesized using acrylamide (AA) as the functional monomer and 2,2-bis(hydroxymethyl)butanol trimethacrylate (TRIM) as the crosslinker. The affinity and selectivity were evaluated by liquid chromatography, and the binding sites and the dissolution constants were measured by frontal chromatography. Compared with the AA–co-ethyleneglycol dimethacrylate (EDMA) MIP, the anti-quercetin AA–co-TRIM MIP exhibited stronger binding and possessed improved column efficacy. A linear plot of the peak area versus sample size (in the range of 0.4–2.2 μg) was obtained, which made it promising for the MIP columns to be directly used for analysis. Before MIP-SPE of the sample of plasma, several washing solvents were tested and it was shown that the careful choice of the right washing solvent is the key step to successful sample extraction. The anti-quercetin AA–co-TRIM polymer selectively extracted quercetin, the effective component in the plasma of rats fed the hydrolyzed extract of *Gingko biloba* L. The recovery (67%) for MIP-SPE was calculated using spiked plasma. The results of the present work showed that the properties of MIP could be improved by modifying the polymerization and that MIP-SPE could be used for direct clean-up of biological samples for the analysis of functional components in vivo originating from an extract of medicinal herbs.

Keywords: Molecularly imprinted polymers; Solid-phase extraction; 2,2-Bis(hydroxymethyl)butanoltrimethacrylate; Quercetin

1. Introduction

Molecularly imprinted polymers (MIPs) are stable synthetic polymers possessing selective molecular recognition sites, which are obtained by using high amounts of crosslinking monomers in the presence of a template molecule. Once the template molecule is removed, specific sites are left behind which are complementary to the template in terms of size, shape and functionality. So ideally, the resulting MIP selectively rebinds the template in preference to other closely related structures. MIPs are referred to

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1570-0232/03/$ – see front matter
doi:10.1016/S1570-0232(02)00796-1

as antibodies as some of them even have selectivities and affinity constants comparable with those of immunoaffinity phases [1–3]. In terms of sample load capacity, stability as well as ease, speed and reproducibility in preparation, MIPs are more advantageous than bioaffinity adsorbents. By designing, the performance of the MIPs can be further improved, as desired [4–7].

Up to now, MIPs have been used in several analytical techniques, including liquid chromatography [8,9], capillary electrophoresis and capillary electrochromatography [10], solid-phase extraction and immunoassay [1,11–15]. Among them, the highly selective MIP solid-phase extraction (MIP-SPE) of analytes that are present in low concentrations or in a complex matrix is the most widely investigated. An increasing interest is seen in the potential application of MIP-SPE in pharmaceutical [16–18] or environmental analyses [19–21]. In these cases, MIP-SPE led to selective enrichment and clean-up of the analytes to levels not achievable with existing methods, and thus a higher accuracy or a lower detection limit in the subsequent chromatographic quantification was obtained. However, a limitation exists when MIP-based SPE is used in aqueous samples such as biofluids, since MIP preparation entails the use of organic solvents, which causes rebinding of the imprints. If the sample first had to be extracted into an organic solvent and only then be applied to the MIP [22,23], the added complication of the method would significantly reduce its attractiveness compared to alternative methodologies. Therefore, investigating protocols that allow the direct application of aqueous samples to the MIP cartridge but then exploit the enhanced selectivity that can be achieved using organic solvents for elution are more advantageous.

In this study, as an example, we explored the application of MIP-SPE to the analysis of functional components in vivo originating from extracts of a medicinal herb, which is an important in the study of the pharmacokinetics of traditional Chinese remedies. Biofluid from animals fed with herbs are generally more complex than that from animals fed with regular drugs since there are various components in herbs. Therefore, to eliminate matrices and other interferences in such pharmaceutical analyses, a prior SPE step is often employed and new selective SPE strategies are called for.

Recently an anti-quercetin MIP with ethyleneglycol dimethacrylate (EDMA) as the crosslinker was successfully prepared and it proved to be able to extract flavonoids with specific pharmacophoric features directly [24]. However, in order to investigate the straightforward MIP-SPE in selective extraction of quercetin, a drug effective component in EGB, from the complex sample of plasma of rats fed with the hydrolyzed EGB, another anti-quercetin MIP of evident hydrophobic polymeric matrix was prepared with TRIM as the crosslinker. Compared with the EDMA–MIP, the TRIM–MIP was evaluated before it was applied in MIP-SPE.

2. Experimental

2.1. Materials

Acrylamide (AA), ethyleneglycol dimethacrylate (EDMA), azobisisobutyronitrile (AIBN), quercetin, chrysin, and naringenin were bought from Acros Organics (Geel, Belgium). Isorhambin and rutin were products of National Medicinal Company (China) (Fig. 1). 2,2-Bis(hydroxymethyl)butanol trimethacrylate (TRIM) was purchased from Aldrich (Steinheim, Germany). 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. Tetrahydrofuran (p.a., THF) was dried by sodium and distilled. Methanol and acetonitrile were HPLC grade; glacial acetic acid and dichloromethane were analytical grade. The water used in HPLC was demineralized and purified by a Millipore system. The other chemicals were used as obtained.

Phosphoric acid solution was prepared by dissolving 3.3 g H_3PO_4 (analytical grade) in 1.0 l H_2O.

The extract of *Gingko biloba* L. (EGB) (Beijing Jinke Green Biotechnology Cooperation, China) was hydrolyzed [25] before it was fed to rats.

2.2. Polymer preparation

Details of polymer synthesis have been previously described [24]. Ingredients of the prepolymerization mixture are shown in Table 1. The polymerization was carried out for 24 h in 60 °C waterbaths.

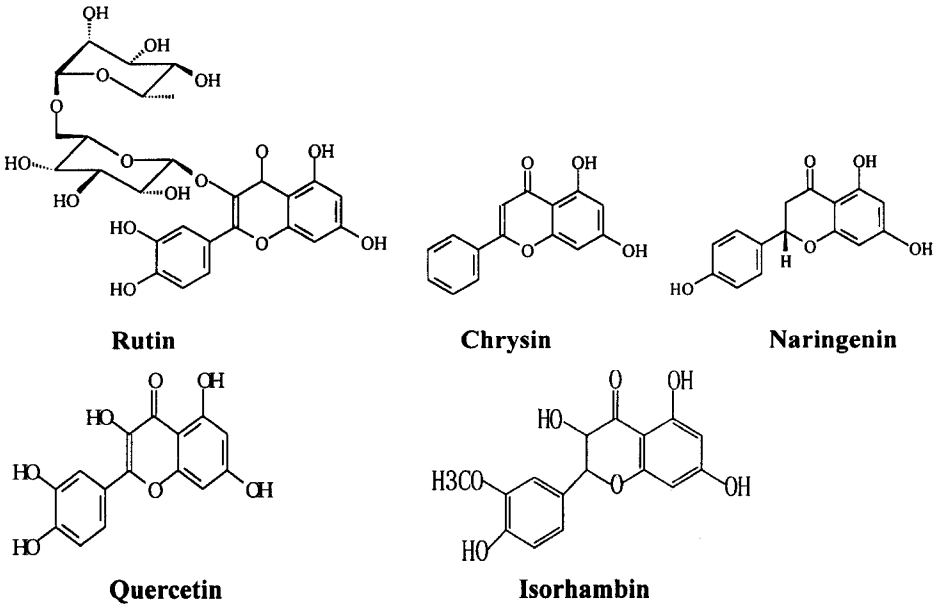

Fig. 1. Structures of the molecules involved in this paper.

Following grinding, polymer particles less than 30 μm were collected, sedimented in acetone and dried under vacuum. Blank polymers (BP) were prepared using the same reaction mixture but without the template. The prepared polymer (MIP or BP) was dry packed into stainless steel HPLC columns (150× 4.6 mm I.D.), and then they were washed with methanol, methanol–acetic acid (9:1, v/v), methanol, methanol–triethylamine (9:1, v/v) and finally methanol to remove the template. Some of the columns were used for the chromatographic test, while others were emptied to prepare the polymer for SPE use.

2.3. Liquid chromatography

A HP1100 HPLC system consisted of a quaternary pump, a variable wavelength detector, an online vacuum degasser and a 20-μl manual injector (Hewlett-Packard, Palo Alto, CA, USA). For a comprehensive selectivity and affinity study, 20 μl of 0.1 m*M* solutions of the template quercetin, and the reference compounds—rutin, chrysin, naringenin and isorhambin—were injected separately onto the columns using methanol as the mobile phase.

The flow-rate was 1 ml/min and the UV detection was set at 254 nm. The retention factor for each analyte was calculated as $k = (t - t_0)/t_0$ where t is the retention time of the sample and t_0 is the retention time of the void marker (acetone). The imprinting factor (IF) was defined as the ratio of the capacity factor of each analyte on the MIP column to that on the BP column. The separation factor (α) was derived from the equation: $\alpha = k$ (quercetin)/k (a reference compound).

Table1
Ingredients of the MIPs in the prepolymerized mixture

MIP	Crosslinker	Monomer	Porogen	Template	Initiator
AA–co-EDMA	EDMA	AA	THF	Quercetin	AIBN
	40 mmol	4 mmol	9 ml	0.8 mmol	20 mg
AA–co-TRIM	TRIM	AA	THF	Quercetin	AIBN
	26.7 mmol	4 mmol	9 ml	0.8 mmol	20 mg

2.4. Binding capacity

The polymer particles were packed into HPLC columns (10×2.1 mm). The dissociation constants and the number of binding sites of the MIP were determined as described previously by frontal chromatography [26]. The mobile phase was methanol with a flow-rate of 0.2 ml/min. A 2-ml volume of quercetin in methanol with concentrations of 1, 2, 3, 4 or 5 m*M* was injected separately using a 2-ml loop. The UV detection was set at 365 nm. The column temperature was ambient. The tests were performed using acetone for determination of the void volume (V_0). The number of binding sites (L_t) and the dissociation constant (K_{diss}) was calculated from the equation

$$\frac{1}{[S]_0(V-V_0)} = \frac{K_{diss}}{l_t} \cdot \frac{1}{[S]_0} + \frac{1}{l_t}$$

where $[S]_0$ is the concentration of the substrate and V is the elution volume of the substrate. $1/[S]_0(v-v_0)$ was plotted versus $1/[S]_0$. The L_t could be calculated from the intercept on the ordinate $1/L_t$, and the K_{diss} could be calculated from the intercept on the abscissa $(-1/K_{diss})$.

2.5. Determination of quercetin and isorhambin in plasma

Plasma was taken from rats fed with 0.40 g of the hydrolyzed EGB 4 h later. Before analysis, the sample was pretreated as follows [27]. A BondElut C_{18} cartridge (500 mg) was preconditioned with 5 ml methanol followed by 5 ml of 10 m*M* acetic acid. Plasma (0.1 ml) was diluted to 1 ml with 10 m*M* acetic acid and then was applied. The cartridge was washed with 10 m*M* acetic acid (3 ml) and then eluted with 6 ml methanol. The eluents were combined and evaporated to dryness under vacuum. The residue was reconstituted into 0.1 ml mobile phase for analysis by HPLC.

The HPLC system consisted of an analytical column (Zobax Eclipse XDB-C_8 4.6×150 mm I.D., Hewlett-Packard, Palo Alto, CA, USA) with a guard column (Dikma). The mobile phase was acetonitrile–phosphoric acid solution (0.33%) (25:75, v/v). The flow-rate was 1.0 ml/min. The wavelength was set at 365 nm. The column temperature was ambient.

2.6. MIP-SPE procedure

The choice of washing solvents was conducted as follows. A 100-mg amount of AA–co-TRIM MIP or AA–co-TRIM BP was dry-packed into a 6-ml empty polypropylene cartridge fitted with a syringe connector. The cartridge was rinsed with 5 ml solvent, which was to be used for wash in a further step (dichloromethane or acetonitrile or methanol). Afterwards, it was conditioned with 5 ml methanol. A 10-μl volume of the prepared methanol solution, in which the concentrations of quercetin and isorhambin were 300 μg/ml separately, was applied slowly onto the cartridge, which was dried with nitrogen. The cartridge was washed slowly with nine portions (0.5 ml) of dichloromethane, acetonitrile or methanol. The effluent liquids at each wash step were collected and analyzed by HPLC as described in Section 2.5.

To treat the plasma sample, a 0.21-g amount of AA–co-TRIM MIP was dry-packed into a 6-ml empty polypropylene cartridge fitted with a syringe connector. The cartridge was conditioned with 5 ml dichloromethane, 5 ml acetonitrile, 5 ml methanol, 6 ml methanol–water (1:1, v/v) and finally 6 ml 10 m*M* acetic acid solution. A 0.1-ml volume of plasma diluted to 0.6 ml with 10 m*M* acetic solution was applied. The cartridge was washed with 2 ml 10 m*M* acetic acid solution, and then washed with 3 ml water. After it was dried by nitrogen, the cartridge was washed with three aliquots (0.5 ml) of dichloromethane and then five aliquots (0.2 ml) of acetonitrile. Finally it was eluted with 4 ml methanol–acetic (9:1,v/v). The eluent was reduced and reconstituted into 0.1 ml mobile phase for analysis as described in Section 2.5.

To calculate the recovery, 100 μl of methanol solution, in which the concentrations of quercetin and isorhambin were 496 μg/ml and isorhambin 320 μg/ml separately, was spiked into 3.9-ml blank plasma (taken from the control rats), so that the concentrations of quercetin and isorhambin in the blank plasma were 12.40 μg/ml and 8 μg/ml separately. The spiked blank plasma was then extracted by the above MIP-SPE procedure followed

by HPLC measurement. The recovery of quercetin was defined as the ratio of the amount extracted to that originally spiked.

3. Results and discussion

3.1. Evaluation of the MIP

The MIP would be used as SPE sorbent to directly extract functional components originating from the herb in plasma. As most MIPs are made using organic solvents as the porogen, in aqueous media polar interactions become weak while hydrophobic interactions become strong. Consequently the aqueous sample can be applied onto MIPs bearing hydrophobic property in reversed-phase type. The log *P* value of TRIM was larger than that of EDMA, which was computed using the method proposed by Crippen et al. [28]. Therefore, the crosslinker TRIM was utilized in order to obtain MIP with hydrophobic features. To gain more insight into the origin of the recognition properties of the imprinted polymers, both the AA–co-TRIM MIP and the AA–co-EDMA MIP were synthesized, keeping the unsaturated chemical bonds in the prepolymerized mixtures constant (Table 1). By comparison, it was proved that chromatographic performances of the reprepared EDMA–MIP column were similar to those of the previously prepared columns [24].

Methylacrylic acid (MAA)–co-TRIM or AA–co-TRIM polymers have been investigated, but there is disagreement concerning the influence of TRIM on the properties of the MIPs [29,30]. However, in accordance with the observations of Kempe [29], the anti-quercetin MIP showed enhanced binding and improved column efficacy when the crosslinker TRIM was used instead of EDMA, which might result from the relatively more open structure of the AA–co-TRIM polymer [31,32].

Table 2 shows that the retention factors and the IF values of all the examined molecules on the TRIM–MIP were much larger than those on the corresponding EDMA–MIP, indicating that imprinting was reinforced when the crosslinker TRIM was employed. However, compared to the AA–co-EDMA MIP, selectivity for the AA–co-TRIM MIP did not improve in terms of the separation factor (α). In fact, the α value of the MIP is correlated with the specificity of the sites induced in the imprinting step. Since the template quercetin bears more than three functional groups interacting with the functional monomer AA, several complexes between quercetin and AA may be formed, causing the anti-quecetin MIPs to possess heterogeneous binding sites. Moreover, Table 2 shows that the larger size of rutin made it exhibit small retention on the quercetin-induced AA–co-EDMA MIP [24], whereas increased ''cross-reactivity'' on the AA–co-TRIM MIP occurred for rutin. This makes sense as TRIM has previously been reported to give rise to polymers with macroporous structure under some conditions [31], which might decrease the steric hindrance when rutin is bound to the anti-quercetin AA–co-TRIM MIP.

Indeed, for this TRIM–MIP column, the shape of the chromatographic peaks was considerably improved. With methanol as eluent the chromatographic peak shapes appeared narrower and exhibited less tailing (Fig. 2). When the sample concentration was in the range of 22–110 μg/ml (the injected volume was set at 20 μl), the retention time was nearly constant and the correlation between the sample size and the peak area was almost linear (Fig. 3). This is promising for MIP columns to be directly used for

Table 2
Chromatographic parameters of the AA–co-TRIM MIP and the AA–co-EDMA MIP

	Quercetin	Chrysin	Naringenin	Rutin	Isorhambin
k (TRIM)	48.6	20.25	22.1	18.0	32.1
k (EDMA)	7.3	2.3	2.1	0.52	5.20
IF (TRIM)	18.7	7.88	6.50	11.25	10.0
IF (EDMA)	3.65	0.83	1.78	0.83	2.81
α (TRIM)	1	2.4	2.20	2.71	1.51
α (EDMA)	1	3.17	3.48	14.04	1.40

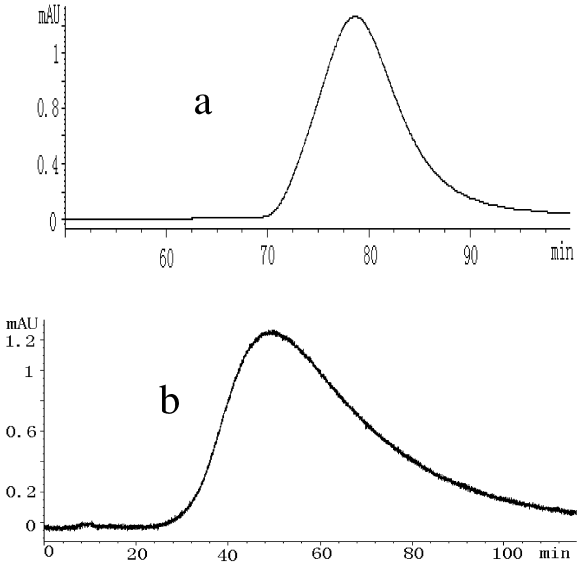

Fig. 2. Chromatogram of quercetin on the anti-quercetin AA–co-TRIM column (a) or on the anti-quercetin AA–co-EDMA column (b) in the eluent methanol with the flow-rate of 1 ml/min. The detection was set at 365 nm. The sample volume was 20 μl with a sample concentration of 0.1 m*M*.

analysis. When using MIPs in HPLC, one common problem is that the more retarded peak is normally very broad, highly asymmetric and tailing badly, which not only can make analysis on a MIP column time-consuming but also makes it difficult to measure the chromatographic parameters accurately.

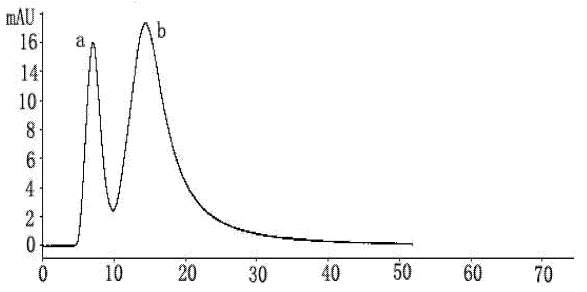

Fig. 4. Separation of isorhambin (a) from quercetin (b) on the anti-quercetin AA–co-TRIM column, the eluent was CH_3CN–H_2O–HAc (97:2:1, v/v/v) with a flow-rate of 1 ml/min, the detection was set at 365 nm. The sample injected contained 3 μg quercetin and 4.5 μg isorhambin.

When adding competing ligands to the mobile phase, peak symmetry became much better. Fig. 4 shows that quercetin could be partly separated from its close analogue isorhambin in acetonitrile–water–acetic acid (97:2:1, v/v/v) on the TRIM–MIP column, even though retention factors sharply decreased due to the disruptive effect of acetic acid and water in the mobile phase on the binding of the analytes.

To shed light on the strength of the MIPs' binding, the number of binding sites and the dissolution constants were determined by affinity chromatography using methanol as eluent (Fig. 5). The AA–co-TRIM MIP had 34.1 μmol sites/g polymer with a dissociation constant of 7.9 m*M*, while the AA–co-

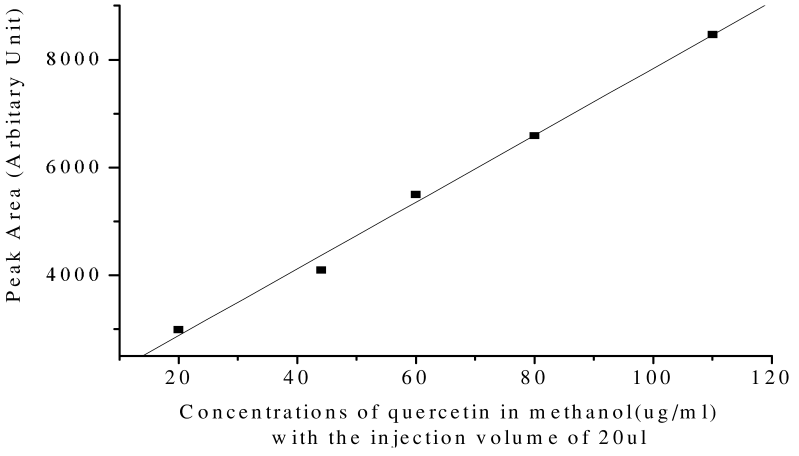

Fig. 3. Linear plot for the peak areas (arbitrary unites) versus the sample size on the anti-quercetin AA–co-TRIM column in the eluent methanol with the flow-rate of 1 ml/min. The detection was set at 365 nm.

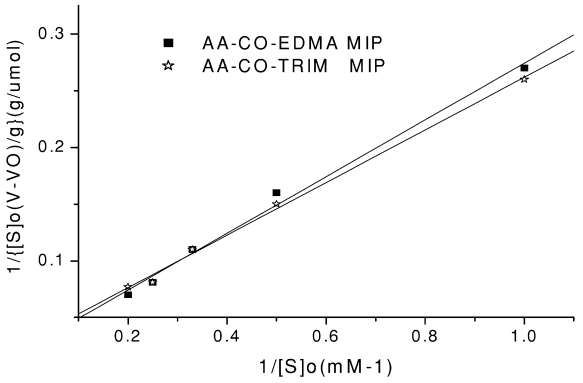

Fig. 5. Plot of $1/[S]_0(V-V_0)$ versus $1/[S]_0$ for the TRIM–MIP and the EDMA–MIP. The number of binding sites (L_t) (calculated from the intercept on the ordinate) was 34.11 μmol/g (TRIM–MIP) and 41.37 μmol/g (EDMA–MIP) separately. The dissociation constant (K_{diss}) (calculated from the intercept on the abscissa) was 10.34 m*M* (EDMA–MIP) and 7.93 m*M* (TRIM–MIP), respectively.

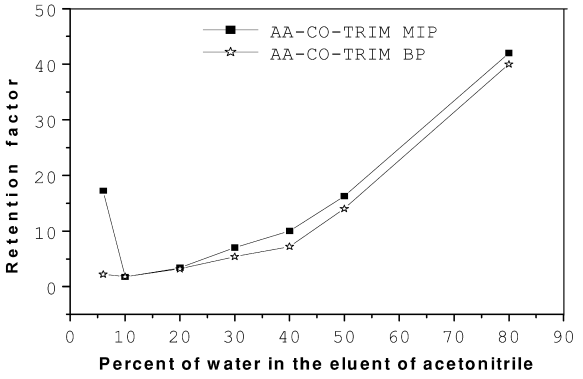

Fig. 6. Retention factors of the AA–co-TRIM column at increasing ratios of water in the eluent acetonitrile. The flow-rate was 1 ml/min. The detection was set at 365 nm. The injected sample was quercetin with the concentration of 0.1 m*M* and the volume of 20 μl.

EDMA MIP had 41.3 μmol sites/g polymer with a dissociation constant of 10.3 m*M*. The smaller dissociation constant implied that the binding of quercetin on the AA–co-TRIM MIP was stronger. However, the numbers of binding sites of the TRIM MIP was not larger than that of the EDMA MIP. This was reasonable considering that the sites studied for MIP by affinity chromatography are the sum of all sites (both specific and nonspecific) [26] and the method is limited by the sensitivity of the spectrometric detection of the HPLC system. Maybe the number of binding sites for the TRIM MIP would be larger than that for the EDMA MIP if only certain specific sites in the MIPs could be dealt with.

As the TRIM–MIP was to be used in plasma, its properties in an aqueous mobile phase were of interest. With acetonitrile as eluent, the quercetin was much more strongly bound to the imprint based on hydrogen bonding, as it could not be eluted in 3 h. Upon increasing the proportion of water in acetonitrile, the retention factor was decreased (Fig. 6). This makes sense as water can interfere with the hydrogen-bonding interactions between amides of the MIP and the phenolic groups of quercetin. However, at high content (more than 50%) of water in the eluent, the MIP exhibited strong hydrophobic interaction due to the high polarity, and quercetin could not be eluted when water was utilized as the eluent. In fact, the hydrophobic matrix of the TRIM–MIP made it possible for the analytes in the plasma to be adsorbed in the reversed-phase mode.

3.2. MIP-SPE

SPE is continuously growing in importance, and is currently a routine sample preparation technique employed in numerous bioanalytical applications. The extent of sample pretreatment will affect the latter analysis, and is especially of significance when analyzing functional components derived from a medicinal herb in the complex sample of biological matrices for the study of the pharmacokinetics of traditional Chinese remedies. In order to investigate the straightforward MIP-SPE process, firstly the sample of plasma was analyzed in the usual way [27]. The concentrations of quercetin and its metabolite isorhambin in the sample of plasma (taken from rats fed with EGB 4 h later) were determined and they were 12.40 and 8 μg/ml, respectively. However, some interferences were still retained in the subsequent chromatographic analysis after the sample was pretreated by the C_{18}-SPE, since adsorption of the analytes on C_{18} were based on the nonspecific reversed-phase interactions (Fig. 7). Apparently, MIP sorbent of specific affinity and selectivity is of need. In fact, quercetin is a functional compound in EGB while its metabolite isorhambin possesses no prominent drug effect [33,34]. Therefore it was of

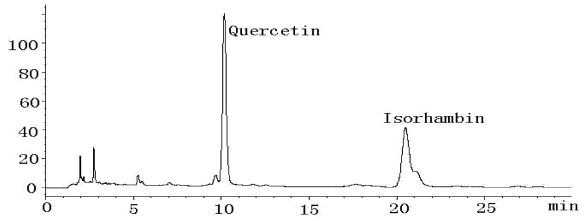

Fig. 7. Chromatogram of the plasma from the rats fed with 0.40 g of the hydrolyzed EGB 4 h later on an analytical column (Zobax Eclipse XDB-C_8 4.6×150 mm I.D.) with the sample pretreated by a C_{18} cartridge. The mobile phase was acetonitrile–phosphoric acid solution (0.33%) (25:75, v/v). The flow-rate was 1.0 ml/min. The wavelength was set at 365 nm. The column temperature was ambient.

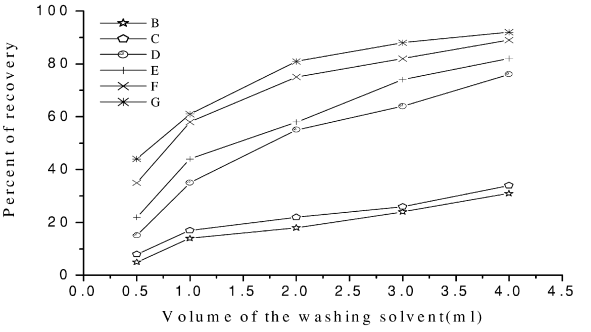

Fig. 8. Percent of recovery versus the volume of the washing solvent on the AA–co-TRIM BP cartridge. (B) (Quercetin) and (C) (isorhambin): washed with CH_2Cl_2, respectively; (D) (quercetin) and (E) (isorhambin): washed with CH_3CN, respectively; (F) (quercetin) and (G) (isorhambin): washed with CH_3OH, respectively.

interest for the MIP to selectively extract quercetin from the plasma.

Before the MIP-SPE, three washing solvents (acetonitrile, dichloromethane and methanol) with different polarities were investigated in order to achieve the selectivity and recovery of quercetin. Dichloromethane is a solvent frequently used in MIP-SPE to disrupt the nonspecific adsorption of low polar matrix components [35,36]. Acetonitrile or methanol is the solvent in which the MIP exhibited high retention for quercetin by the chromatographic evaluation. Before application, the polymer was sufficiently conditioned with the solvent to be used for washing. As the selection of solvent was for the MIP-SPE wash step, the polymer was dried after the sample was applied to imitate the later SPE process. The wash was conducted in several steps with every fraction collected and analyzed. The percentage of cumulative recovery relative to the total eluted amount was plotted against the volume of the solvent used. The resulting graphs are presented in Figs. 8 and 9.

As was previously reported, a small difference between the elution profiles of the MIP and those of the BP was observed [37]. Figs. 8 and 9 show that quercetin and isorhambin could only be weakly eluted by dichloromethane whether from the BP or from the MIP. When 1 ml dichloromethane was used, almost no quercetin or isorhambin was eluted. When the volume of dichloromethane was as high as 4 ml, the recovery of quercetin or isorhambin for both the MIP and the BP was not more than 30%. This indicated that the binding of quercetin and isorhambin on the polymers was based on hydrogen-bonding interactions and dichloromethane weakly destroys such interactions. Figs. 8 and 9 also show that most of both quercetin and isorhambin were recovered from the MIP or the BP with only small amount of the polar solvent methanol, yet quercetin could not be selectively extracted in preference to isorhambin.

When the less polar solvent acetonitrile was used, although the MIP showed a high affinity for quercetin's close analogue isorhambin, it was more inclined to bind the template quercetin. The recovery of isorhambin was markedly more than that of quercetin if <1 ml acetonitrile was used. In contrast, the difference of recovery between quercetin and

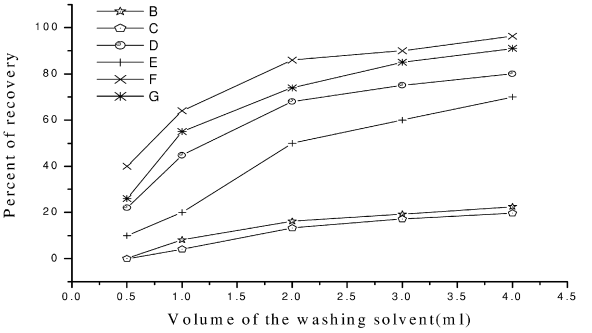

Fig. 9. Percent of recovery versus the volume of the washing solvent on the AA–co-TRIM MIP cartridge. B (Isorhambin) and C (Quercetin): washed with CH_2Cl_2, respectively; D (Isorhambin) and E (Quercetin): washed with CH_3CN, respectively; F (Isorhambin) and G (Quercetin): washed with CH_3OH, respectively.

isorhambin for the corresponding BP was <10% under similar conditions because adsorption on the BP was based on nonspecific interactions. From the above test, it could be concluded that selective extraction of quercetin with the removal of isorhambin from the sample of plasma was possible by the MIP-SPE if acetonitrile was employed at the SPE wash step.

At present, SPE by MIPs is widely investigated. However, the evident selectivity for MIP in organic solvents might represent a serious limitation of the technique, since the majority of biological samples are aqueous in nature. Therefore in some reported examples of MIP-SPE, a circumvent protocol was exploited, that is, the samples first had to be extracted into an organic solvent and then the MIP-SPE was employed to deal with the organic extract [22,23]. Apparently the added step would significantly reduce its attractiveness compared with alternative methods. As in aqueous media the analytes can be strongly adsorbed based on MIP's hydrophobic property, the sample of plasma was directly applied onto the TRIM–MIP cartridge. The adsorption conditions were then tuned based on the above optimizing procedure to make the SPE cartridge selectively trap quercetin whereas other matrix components are not retained.

Before application, the plasma was adjusted to pH 6 in case quercetin was ionized. After the sample was applied, the cartridge was washed with water to remove the hydrophilic components such as proteins or saccharides in the plasma sample. Since the water content in acetonitrile reduced the retention time of quercetin on the TRIM–MIP, the cartridge needed completely drying before the wash step in case quercetin would be removed when washing the cartridge. Besides acetonitrile, dichloromethane was employed at the wash step to eliminate some unknown weak polar interferences derived from EGB. In fact, matrix peaks in the chromatogram could still be observed if dichloromethane was the default at the wash step. However, if an additional amount of acetonitrile were used to further eliminate the residual matrix peaks, the recovery of quercetin would decrease.

A chromatogram of the sample of plasma after the MIP-SPE is shown in Fig. 10. Quercetin was extracted while almost no matrix peaks existed. To check the recovery, the blank plasma was spiked at concentrations of quercetin and isorhambin of the same level as those in the experimental plasma (quercetin and isorhambin were 12.40 and 8 μg/ml, respectively). The spiked sample was treated using a similar MIP-SPE process and then analyzed by HPLC. The recovery of the spiked quercetin was calculated by three repeated results and it was 67% (RSD<4%). Notably there was no peak at the retention time of the analyte when the nonspiked blank plasma was tested, which confirmed that there was no noticeable bleeding of the template quercetin in the MIP-SPE procedure.

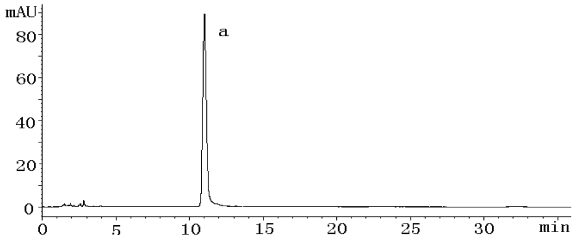

Fig. 10. Chromatogram of the plasma from the rats fed with 0.40 g of the hydrolyzed EGB 4 h later on an analytical column (Zobax Eclipse XDB-C_8 4.6×150 mm I.D.) with the sample pretreated by an anti-quercetin AA–co-TRIM cartridge. Peak (a) corresponds to quercetin. The mobile phase was acetonitrile–phosphoric acid solution (0.33%) (25:75, v/v). The flow-rate was 1.0 ml/min. The wavelength was set at 365 nm. The column temperature was ambient.

4. Conclusions

To extract functional components in the plasma of rats by MIP-SPE fed a medicinal herb, an anti-quercetin polymer with a hydrophobic matrix was prepared using TRIM as the crosslinker. The results presented here indicate that the AA–co-TRIM MIP was superior. It exhibited stronger binding for quercetin and possessed improved column efficacy. Though the TRIM–MIP exhibited affinity for the template quercetin as well as its structural analogue isorhambin, careful choice of the washing solvents in the MIP-SPE could remove isorhambin and the other matrix components from the sample of plasma, allowing a specific extraction of the drug effective component quercetin feasible. The results demonstrate that MIP-SPE is applicable for the analysis of

functional components derived from herb in the complex sample of plasma.

Acknowledgements

The project is supported by National Natural Science Foundation of China (NSFC) 29992590-2.

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