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Affinitive Separation and On-Line Identification of Antitumor Components from *Peganum nigellastrum* by Coupling a Chromatographic Column of Target Analogue Imprinted Polymer with Mass Spectrometry

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A coupled LC−MS (liquid-phase chromatography and mass spectrometry) system consisting of a combination of a column of molecularly imprinted polymer (MIP) and a MS detector was used for affinitive separation and on-line identification of the antitumor components, harmine and harmaline, from the methanol extract of *Peganum nigellastrum* seeds. Three molecularly imprinted polymers were synthesized with porogens bearing different hydrogen bonding capacities with harman, the structural analogue of harmaline, and harmine as the template. The affinity and selectivity of the anti-harman MIPs for the targets, harmine and harmaline, were investigated chromatographically, and the influences of the porogens and sample loads on the retention of the target compounds were also discussed. In addition, the target binding capacities of the MIPs were evaluated by frontal chromatography. When the MIPs were further used in a LC−MS system to separate the extract of herb, it was observed that imprinting with different porogens would cause the MIPs to exhibit different tendencies to adsorb the matrix components from the herb. Though the MIP prepared with a porogen of less hydrogen bonding capacity possessed higher selectivity and stronger affinity for the targets, matrix components in the herb extract interfered with the chromatographic performance more seriously when it was used as the LC solid phase in the LC−MS system for selective extraction of harmaline and harmine from the crude herb extract. Positively, the MIPs were stable and reproducible in the separation test, and the imprinting columns could efficiently separate the antitumor components from the herb extract after the sample was simply pretreated. The work in this paper would be helpful for the further extraction and identification of certain pharmacophoric compounds in herbs by a LC−MS system using MIPs as the HPLC solid phase.

Molecular imprinting has been recognized as a technique for the ready preparation of polymeric materials containing recognition sites of predetermined specificity. The principle of the technique involves the formation of definable interactions between a given template molecule and polymerizable functional monomers during polymerization with excess cross-linking agents. Subsequent removal of the template from the resulting polymer yields complementary binding sites (imprints), which consist of functional groups with a particular arrangement fit for the corresponding templates. At present, MIPs have been successfully prepared for many kinds of compounds, including amino acid derivatives,[1−4] drugs,[5,6] sugars, and sugar derivatives.[7,8] Furthermore, polymers having an affinity for the target compounds were also prepared by using structural analogues of the targets as templates.[9−11] This is indeed an operable approach for overcoming the limits of the present imprinting protocols when employing the target compound itself as the template to prepare the corresponding MIP is problematic because the target compound is poorly soluble in weak polar organic solvent, expensive, or difficult to obtain.

Up to now, several applications of MIPs have been extensively studied. MIPs as stationary phases in high performance liquid chromatography (HPLC) for the enantiomeric separation of drugs have been an attractive focus, and several intriguing separations resulting in high separations and resolutions have been performed.[6,12,13] In addition, separation approaches combining thin-layer chromatography (TLC) with molecular imprinting have been

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reported.[14−16] Various examples of the application of MIPs as solid-phase extraction (SPE) cartridges for concentrating and purifying the templates[17,18,21] or a class of structurally related analysts in the biofluid samples [19,20] or in environmental waters [11,22−24] have been described in the literatures.

However, the investigation of MIPs as the HPLC solid phase in a LC−MS system for affinitive separation and on-line identification of active components from herbs is scarce. Nowadays, LC−MS is applied widely as an accurate, sensitive, and specific technique for efficient separation and identification of potential active components in traditional Chinese herb extracts. Because of the inherent specificity of MS, not only investigated target compounds can be confirmed from complex interferences in case of without standards, but also various trace unknown coexisting compounds in herb extracts can be easily recognized by their molecular ions, which is favorable for the discovery of lead compounds from herbs. However, an increasing number of new and challenging problems in herb isolation call for novel sorbents that are easy to use and rugged enough to secure proper LC−MS use. At present, solid phases of the reverse-phase type and normal-phase type have been widely employed in HPLC columns for on-line separation and identification of active compounds from complex herb extracts in a LC−MS system. Yet, since those systems utilize nonspecific interactions, LC separation conditions have to be carefully determined according to the chemical characteristics of the target compounds and various other coextracted components from the herb matrix. Moreover, the selected LC conditions should be favorable to LC solid phases and suitable for MS detection. Consequently, in some cases, compromised elution conditions have to be adopted to avoid solid phases' decomposition or to keep the mass spectrometer in proper condition. Therefore, it was advantageous to investigate the application of novel affinity-type solid phases, such as the robust MIPs in a LC−MS system (MIP-LC−MS) for target-selective extraction and identification of components from herb extracts, because separation conditions for such an on−off system usually do not need to be carefully selected, and in most cases, the selected eluents are regular organic solvents that are compatible with the detection of mass spectrometers. More attractively, by applying such a MIP-LC−MS system, a class of structural analogues of specific pharmacophoric features in herbs may be simultaneously trapped and identified in view of the structure−activity correlation principle.

In this study, the feasibility of using a MIP-LC−MS system for selective separation and on-line identification of antitumor components,[25] harmine and harmaline, from the methanol extract of *Peganum nigellastrum* seeds was demonstrated. Three MIPs were prepared in different porogens using harman, the structural analogue of harmaline and harmine, as the template. The selectivity and affinity of the anti-harman MIPs for the targets, harmaline and harmine, were evaluated by the chromatographic mode, and the binding sites of the MIPs were determined by frontal chromatography. Moreover, it was further proven by the MIP-LC−MS test that the porogens' use at the imprinting step would take effect on the adsorption and separation behaviors of MIPs. To our knowledge, this is the first example of using MIPs as a chromatographic solid-phase coupling with MS for selective separation and on-line identification of specific pharmacophoric components from complex herb matrix.

EXPERIMENTAL SECTION

Equipment. A HP1100 HPLC system with a quaternary pump, a variable wavelength detector, an on-line vacuum degasser and a 20 L manual injector (Hewlett-Packard, Palo Alto, CA) and a Mariner time-of-flight (TOF) mass spectrometer (MS) with an ESI (electrospray ionization) interface and a Sciex APCI (Atmospheric Pressure Chemical Ionization) interface (PE PerSeptive Biosystems, Framingham, MA) were used. For ESI ionization, the detailed operating conditions were as follows: The ion polarity was positive; the nebulizer gas and curtain gas were nitrogen with a flow rate of 0.5 and 1.5 mL/min, respectively; the nozzle and quadrupole were both heated to 140 °C; the spray tip and nozzle potential were 5000 V and 100 V, respectively; and the acquisition speed was 3 s/spectrum. When the APCI ionization was used, the nebulizer, curtain, and auxiliary gas were nitrogen with a flow rate of 0.70, 0.90, and 2.5 mL/min separately; the nozzle, quadrupole and spray chamber were heated to 170, 140, and 475 °C separately. The other conditions were the same as those used in ESI.

Materials. Harmine (HMI), harman (HMA), ethylene glycol dimethacrylate (EDMA), methacrylic acid (MAA), and azobis-(isobutyronitrile) (AIBN) were purchased from Acros Organics (Geel Belgium). Harmaline (HML) was bought from Sigma. Before use, EDMA was distilled under vacuum after being extracted with 10% sodium hydroxide brine and dried over anhydrous magnesium sulfate, and AIBN was recrystalized in methanol. Toluene was dried by sodium and then redistilled. Methanol, acetonitrile, and tetrahydrofuran were of HPLC grade; glacial acetic acid was of analytical grade. The water used in the HPLC was demineralized and purified by a Millipore system.

Preparation of MIPs. The template molecule harman (1 mmol) and the functional monomer MAA (4.0 mmol) were dissolved in different porgens (8 mL) (see Table 1) in a test tube, and then the cross linker EDMA (30 mmol) and the initiator AIBN (41.0 mg) were added, followed by an additional amount of porogen (2 mL). After the mixture had been degassed, the tube was sealed, placed in a 60 °C water bath and incubated for 24 h. The rigid polymer obtained was ground in a mortar and sieved to pass through a 35 μm sieve. Fine particles were removed by

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Table 1. Polymers Prepared and Porogens Used in the Corresponding Polymers[a]

polymer	P1 BP1	P2 BP2	P3 BP3
porogen (10 mL)	toulene and MeCN (1:1, v/v)	MeCN	THF

[a] The polymers were prepared as described in the Experimental Section using harman (1 mmol), MAA (4 mmol), EDMA (30 mmol). P1, P2 and P3 were imprinted polymers. BP1, BP2 and BP3 were corresponding blank polymers.

decantation in acetone and then were dried under vacuum. The polymer particles were dry-packed into stainless steel columns (150 mm × 4.6 mm i.d.). Then they were washed on-line with methanol, methanol/acetic acid 8:2 (v/v), and methanol until a stable baseline to remove the template molecules was obtained. As a control, nonimprinted blank polymer (BP) in the absence of the template was also prepared and treated in the identical manner.

High Performance Liquid Chromatography. The HPLC study of the MIP columns was performed isocratically at room temperature. The mobile phase was methanol, the flow rate was 0.5 mL/min, and the sample volume injected was 20 μL. Different sample loads were injected by changing sample concentrations with the injected volume fixed. Detection was carried out using UV absorption at 254 nm. The capacity factor (k') was calculated by the equation $k' = (t_R - t_0)/t_0$, where t_R is the retention time for a sample and t_0 is the time to elute the void marker acetone. The separation factor a was defined as the ratio of the k' value of one molecule on a MIP column to that on its corresponding blank polymer (BP).[32]

Frontal Chromatography. Dissociation constants and the number of binding sites of P1 and P2 were determined by frontal chromatography, as previously described.[26,27] The polymer particles were packed into HPLC columns of 10 × 2.1 mm. The eluent used was methanol with a flow rate of 0.2 mL/min, and the temperature of the column was ambient. Substrates of harman, harmine, and harmaline in different concentrations were applied in a 2-mL loop.

Sample Preparation. For preparation of the solution of herb extract, 2 g of seeds of *P. nigellastrum* were refluxed on a Soxhlet in 40 mL ethanol until the color of the solution was pale red.[28] The solution was reduced to dryness by an evaporator, then the residues were redissolved in 200 mL of methanol. Finally, the solution of herb extract was passed through 0.45-μm filter films for analysis. The concentrations of harmaline and harmine in the solution of the herb extract were determined by HPLC with an analytical column (ZORBAX Extend-C18 4.6 × 150 mm i.d., Hewlett-Packard, Palo Alto, CA) using harmaline and harmine as the external standards. The eluent used in the determination was methanol/8mM triethylamine (1:1, v/v) at a flow rate of 1 mL/min. The sample size for injection was 20 μL, the detection was carried out at 336 nm, and the temperature of the column was ambient. Before analysis in HPLC, the solution of herb extract was analyzed by positive ESI-TOFMS. Moreover, the eluted fractions corresponding to harmine and harmaline in the HPLC analysis were also analyzed by positive ESI-TOFMS.

On-Line Separation and Identification of the Herb Extract. The Mariner time-of-flight (TOF) mass spectrometer is equipped with an ESI ionization source and an APCI ionization source. ESI is a "soft-ionization" source. Heat-labile components in the herb extract can also be detected by ESI-MS. However, for on-line LC−MS use, APCI is advantageous, because it can work well even when LC is used at a regular flow rate. On-line separation of the solution of herb extract (prepared as described above) was performed by directly combining a molecular imprinting column with the APCI-TOFMS detector. The ionization was positive, the mobile phase used in LC was methanol, and the flow rate was 0.5 mL/min. The sample size injected was 20 μL for P1 and 13 μL for P2. The column temperature was ambient. When the sample needed pretreatment, the processing mode was as follows: The crude herb solution (0.5 mL) was slowly filtered through a C18 Bond-Elut (Varian) column that had been conditioned by methanol/8mM triethylamine (2:8, v/v), and then the Bond-Elut was washed with methanol three times. The effluents and the washing-out solutions were merged and reduced under vacuum. The residues were reconstituted in 0.5 mL methanol for use.

RESULTS AND DISCUSSION

Polymer Preparation. The goal was to find a polymer that as the solid phase would retain and resolve the antitumor compounds harmine and harmaline from the crude extract of *P. nigellastrum* seeds. In addition, the capacity of the polymer would be acceptable for separation. However, because harmine and harmaline cannot be sufficiently dissolved in weak polar solvents, anti-harmine or anti-harmaline MIPs cannot be obtained by the present imprinting protocals. Therefore, the MIPs were prepared using harman, the structural analogue of harmine and harmaline as the template. Following the literature on preparing polymers possessing high affinity and selectivity for nitrogen bases, MAA was chosen as the functional monomer. Three porogens, which could dissolve the prepolymization mixture well, were used in the polymerization process. The porogens were the mixed solvent (toluene and acetonitrile 1:1, v/v) and acetonitrile and tetrahydrofuran separately. Because different porogens can give rise to materials with differences in retention, structure and morphology,[29] the three porogens used in the polymerization step were expected to influence the behaviors of the corresponding polymers in the following tests.

The bulk polymers obtained appeared to be different. The P2 and P3 synthesized in acetonitrile and tetrahydrofuran separately were opaque and hard, whereas the P1 synthesized in the mixed solvent was transparent and crisp. In the decantation step, the lost amount of particles of P1 was considerably more than that of P2 or P3, which may have resulted from the greater swelling of P1.[29] Methanol containing acetic acid was used to remove harman from the MIP columns, because it is an effective solvent for eluting basic templates from the MIPs using MAA as the functional monomers.[30] Because the preparation of the anti-harman MIPs was to efficiently separate harmine and harmaline from the herb extract, the affinity and selectivity of the MIPs for harmine and harmaline were then investigated by the chromatography mode.

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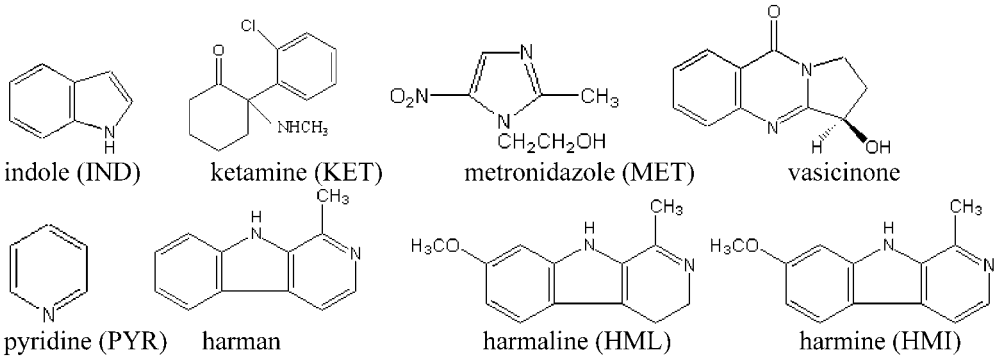

Figure 1. Structures of the molecules described in this paper.

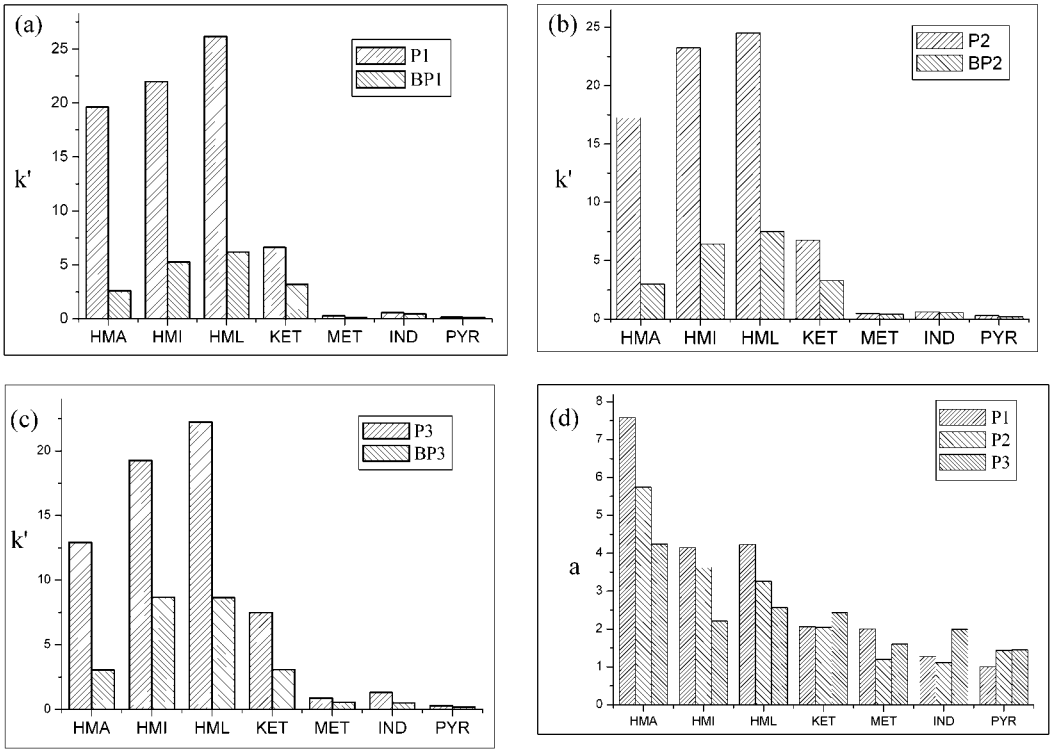

Figure 2. Results from columns packed with various imprinted or blank polymers as described in the Experimental Section. The mobile phase was methanol, the compounds were injected separately, the sample volume was 20 μL, and the concentration was 0.1 mmol L^{-1}. The separation factor, α, was calculated as the ratio of the capacity factor (k') on the imprinted column to k' on the nonimprinted blank column: $\alpha = k'_{imp}/k'_{bl}$.

Chromatographic Evaluation of the Polymers. As we know, the choice of the type of solvent is very critical for the binding and recognition of MIPs. A polar and protic solvent weakens the noncovalent specific interactions between the template and the polymer matrix and, consequently, reduces the affinity and selectivity of the MIP for its template. In the eluent acetonile, the MIPs exhibited a high affinity for harman, harmine, and harmaline, and the three compounds could not be eluted in 3 h. However, methanol was chosen as the mobile phase for characterizing the polymers, because methanol could dissolve the components in the herb extract well and the basic analytes still exist in the native form.

In Figure 2, the capacity factors and separation factors of the analytes injected on the columns packed with the polymers are illustrated. The MIPs exhibited a high affinity to harman as well as its structural analogues, harmine and harmaline, but they possessed only a small retention to the other structurally unrelated compounds. This indicated that the anti-harman MIPs possessed high "cross-reactivity" only for harman's structural analogues, harmine, and harmaline, which made it possible for the MIPs to be used for the selective extraction of the antitumor components from the herb extract. Furthermore, it could be seen that all of the examined molecules were retained longer on the MIPs than on the BPs, suggesting that the adsorbents of larger capacities could be achieved by preparation of imprinted polymers. The capacity factors on the MIPs were increased in the order harman, harmine, and harmaline, which was in agreement with the order in terms of basicity for the three molecules. This is reasonable, considering the basicity of a substrate was also an important factor for recognition in MAA-based molecular imprinting.[31] However, the separation factors of harman on the MIPs were larger than those of harmine and harmaline, because the MIPs were produced by using harman as the template.

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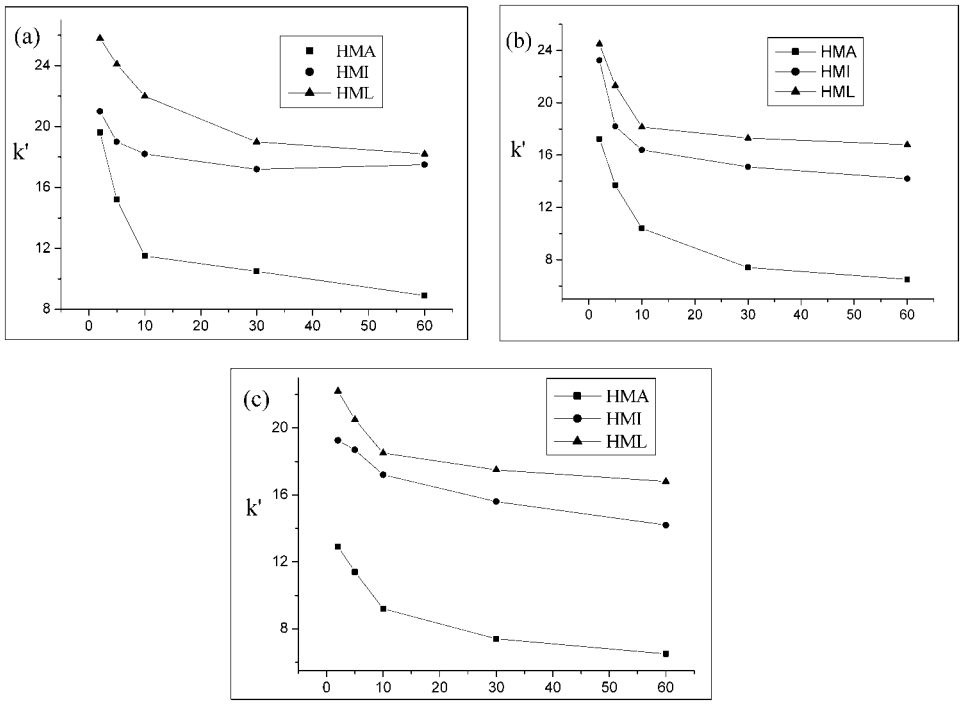

Figure 3. Capacity factor (*k*′) versus sample load (nmol) of HMA (harman), HMI (harmine) and HML (harmaline) on (a) P1, (b) P2, and (c) P3. Flow rate, 0.5 mL/min. The mobile phase was methanol.

The porogens indeed influenced the chromatographic performance of the MIPs. The capacity factors and the separation factors were decreased in the order P1, P2, and P3, which corresponds to the increasing order of the porogens used in the imprinting step in terms of hydrogen bonding capacity: P1 (toluene and acetonitrile), P2 (acetonitrile), P3 (tetrahydrofuran). This makes sense. In fact, the association of harman with MAA is mostly driven by Bronsted acid−base interactions. The stronger the hydrogen bonding capacity of the porogen used in the imprinting step, the stronger the formation of template assemblies was disturbed, and then the more the imprinting efficiency was decreased. Because a porogen (tetrahydrofuran) with stronger hydrogen bonding capacity was used in the preparation of P3, the separation factors on the P3 column were considerably smaller. This implied that the imprints on P3 were not well-formed, and the binding of harman, harmine or harmaline on P3 was mostly based on nonspecific interactions.

As we know, it was very important to study the retention behaviors at several sample loads when characterizing imprinted materials.[29,32] This was also necessary, because the MIPs in our work would be used to separate the crude herb extract. The influence of sample loads on the retention behaviors of the three MIPs is shown in Figure 3. When the sample load was 2 nmol, chromatographic peak shapes on the three polymers appeared broad. As the sample load was progressively increased, the capacity factors of harman, harmine, and harmaline on the three imprinted phases were decreased, and the chromatographic peak shapes became narrower. Such chromatographic dependence of the MIPs on sample loads probably resulted from binding sites

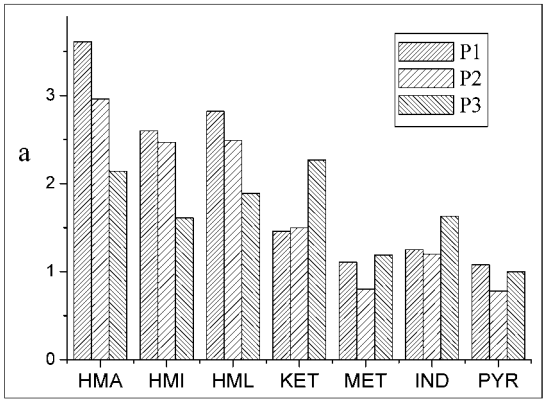

Figure 4. Separation factor (α) of the columns packed with imprinted polymers P1, P2, and P3. The mobile phase was methanol with a flow rate of 0.5 mL/min. The sample injected was harman, and the sample size was 60 nmol.

on the MIPs composed of a small number of high-energy selective binding sites and a large number of weaker less selective binding sites. Moreover, this inhomogeneity could also cause selectivity of the MIPs decreased as the increasing of sample loads.[29] It can be seen from Figure 4 that separation factors on the MIPs become smaller at higher sample loads. When the sample load was as high as 60 nmol, target selectivity on P1 or P2 was still acceptable, but P3 exhibited "cross-reactivity" for most nitrogen-based compounds as a result of its poor imprinting efficiency. Obviously, P3 was not suitable for further use in the separation of the herb extract.

Binding Capacities of P1 or P2 on Target Compounds. Following the above study, the binding capacities of P1 or P2 for harman, harmine, and harmaline were further studied by frontal chromatography in the eluent methanol. The result is shown in Table 2. The number of binding sites (BS) or corresponding

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Table 2. Binding Study for P1 and P2 by Frontal Chromatography in the Eluent Methanol

	binding sites (μmol/g polymer)		dissociation constant (mM)	
substrate	P1	P2	P1	P2
harman	10.0	7.07	4.36	5.48
harmine	14.20	12.97	1.12	4.82
harmaline	14.40	13.16	0.80	4.30

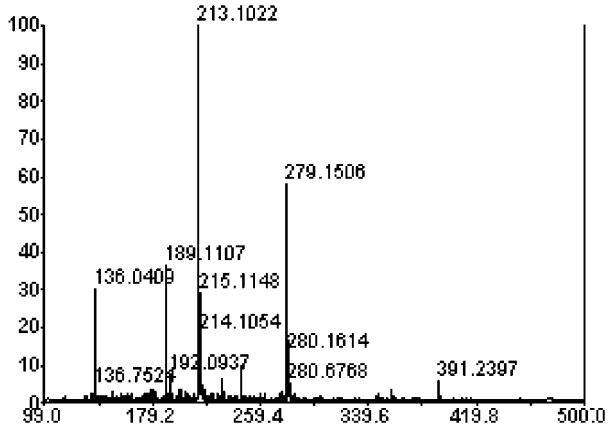

Figure 5. Mass spectra of the herb extract by using ESI-TOFMS.

dissociation constants (DC) was about the same range as reported.[26,27] And in agreement with the k' values illustrated in Figure 2, the BS values of harman on both P1 and P2 were smaller than those of harmine or harmaline; however, a significant difference between the BS values for P1 and P2 was not observed, though the imprinting efficiency on P1 was obviously higher than that on P2, as illustrated in Figure 2. This makes sense. As we know, the imprinting procedure produces sites with various degrees of specificity, because complexes formed before polymerization between the templates and the functional monomers are in a dynamic equilibrium. In fact, the sites determined here by frontal chromatography were the sums of various degrees of specify, and the interactions studied were the averages of all interactions (both specific and nonspecific) existing between the substrates and the polymers.[26]

Analysis of the Sample of Herb Extract. Since the eluent (water−triethylamine) employed in LC is detrimental for MS detection in a positive mode and the TOFMS detector with high response and sensitivity is not suitable for quantity use, harmine and harmaline in the herb extracts were not analyzed by a LC−MS system; however, an off-line mode was adopted. At the beginning, the existence of harmine and harmaline in the herb extract was confirmed by the ESI-TOFMS. Moreover, during analysis of the herb extract in LC, the fractions corresponding to the UV peaks were collected, and then they were further identified by ESI-TOFMS to confirm the peaks corresponding to harmine and harmaline. Concentrations of harmine and harmaline in the solution of herb extract were 150 μg/mL and 82 μg/mL separately, which were calculated by the UV peak areas with harmine and harmaline as the external standards. MS analysis of the herb extract is shown in Figure 5. It could be seen that there were various compounds in the crude methanol extract. The main peaks m/z 213 and m/z 215 were identified as harmine and harmaline,

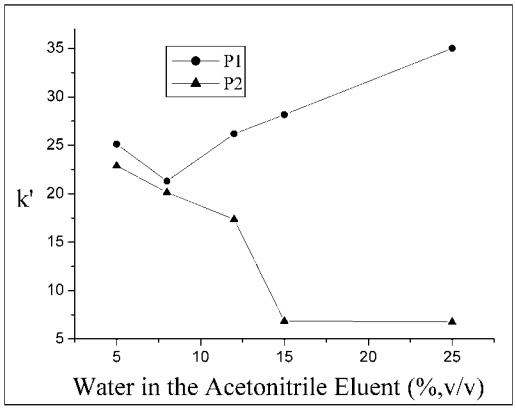

Figure 6. Capacity factors of harman on the columns packed with the imprinted polymers P1 and P2 with varying ratios of acetonitrile/water as the eluent. Flow rate, 0.5 mL/min. The volume injected was 20 μL, and the concentration was 0.1 mmol L^{-1}.

respectively.[33] m/z 183 was not observed, indicating that almost no harman existed in the crude extract.

MIP-LC−MS Test. At present, separation by MIPs is generally investigated in the off-line mode. That is to say, the MIPs are used as SPE cartridges for cleanup of samples before analysis. However, if integrated separation and identification could be achieved by combining a MIP column directly with a MS detector, it was inevitably advantageous. In this study, such an integrated approach was tried by combining the P1 or the P2 column directly with the APCI-TOFMS detector, which can offer higher specificity and sensitivity than other detection systems, such as UV/vis, in identifying components in herb matrix. And the positive ionization was employed in the APCI interface for MS detection, because the nitrogen base compounds, including harmine and harmaline, are very sensitive to this condition.

At the beginning, the MIP columns were tried to directly separate the crude extract. For the P2 column, this attempt was workable, although the shape of the peak in the chromatogram of total ion current was tailing. In contrast, for the P1 column, the peak corresponding to harmaline in the chromatogram of total ion current was very flat shape and could not even be observed. Obviously, harmaline's desorption from the polymer was disturbed by the matrix components from the herb, since such a problem was not observed when harmaline was injected independently. After the above trial, retention behaviors of P1 and P2 were reexamined. It was observed that the retention of the targets on the P2 column were the same before as after it was washed with methanol containing acetic acid; however, the column P1 could not be further used for HPLC, because it possessed a very high backpressure. In fact, P1 and P2 differed only in the porogens used in the polymerization. So the differences exhibited by P1 and P2 in direct separation of the crude herb solution indicated that the nature of the porogens used in the imprinting step was of significance for the MIPs' adsorption when the MIPs were subject to mulitcomponent samples. As we know, the porogens used in the imprinting step for the two polymers possessed different hydrogen bonding capacities and differed in terms of ability to solvate the growing polymer chain when polymerizing. This most likely led to differences in the porous structure and polarity of the pore walls. And consequently, different nonspecific adsorptions and interferences were formed on the two polymers

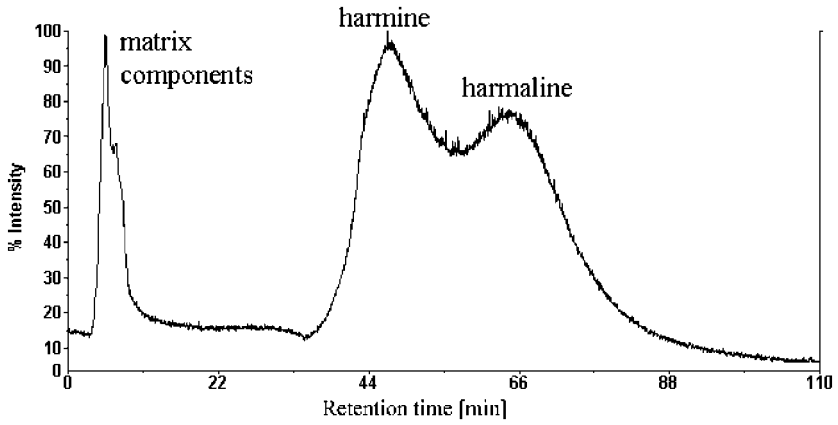

Figure 7. Chromatogram of the total ion current for the LC−MS separation on the column packed with imprinted polymer P1.

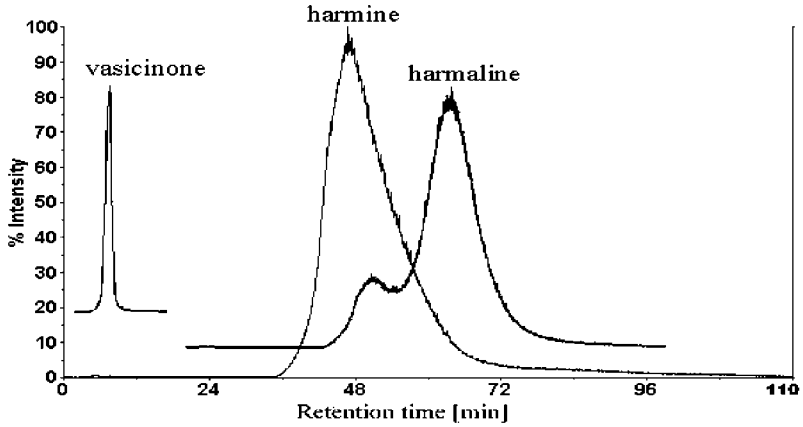

Figure 8. Extracted ion monitoring chromatogram of component vasicinone (*m*/*z* 203), harmine (*m*/*z* 213), and harmaline (*m*/*z* 215) for the LC−MS separation on the column packed with imprinted polymer P1.

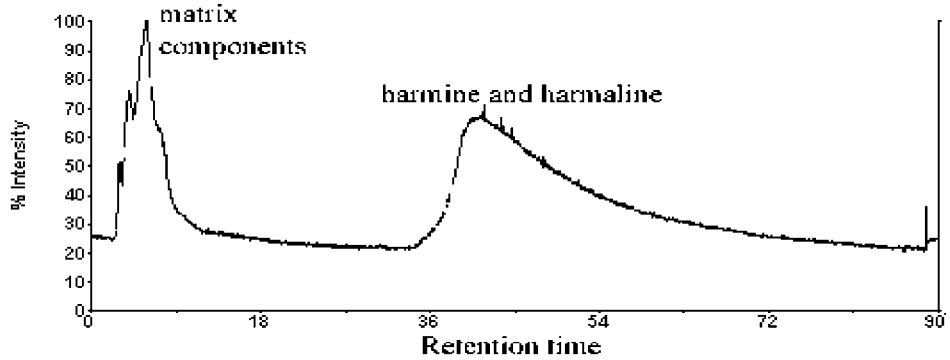

Figure 9. Chromatogram of the total ion current for the LC−MS separation on the column packed with imprinted polymer P2.

when they were used to separate the herb extract. Notably, chromatographic performance for the P1 column still could be recovered after it was treated as follows: The polymer was emptied out, washed with methanol−acetic acid and then methanol in a beaker, and then dried under vacuum and repacked in the column. This further verified the common statement that one advantage of MIPs over classical antibodies is their physical and chemical stability.

Because interferences in the herb extract disturbed the chromatographic performance of the polymers, the LC−MS separation was then investigated with the sample pretreated with C18 Bond-Eluts. The chromatographic peaks corresponding to harmine and harmaline on the P1 column could be explicitly seen after the treated sample was injected. In addition, the chromatographic shape for P2 became better. Apparently, sample pretreatment was necessary when MIPs were used as the chromatographic solid phase to directly separate multicomponent samples. As we know, C18 Bond-Eluts tend to strongly adsorb some low-polar substances from the samples. Therefore, the poor chromatographic performance exhibited by the P1 column in separation of the crude herb solution probably resulted from its nonspecific adsorption of certain low-polar interferences from the herb. The different hydrophobic performance exhibited by P1 and P2 in the eluent acetonile−water may illustrate this point. From Figure 6, it can be found that with the water content slowly increased in the eluent acetonile, the retention of harman on the P1 column or the P2 column was significantly decreased, because water interferes with the Bronsted acid−base interactions between

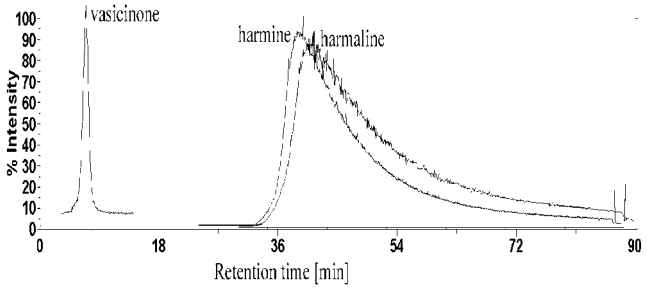

Figure 10. Extracted ion monitoring chromatogram of component vasicinone (*m*/*z* 203), harmine (*m*/*z* 213), and harmaline (*m*/*z* 215) for the LC−MS separation on the column packed with imprinted polymer P2.

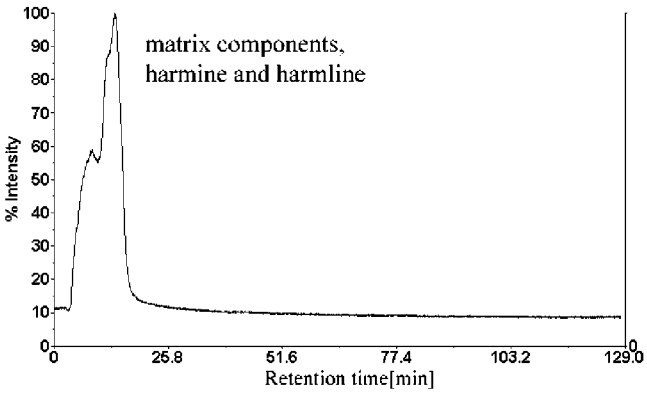

Figure 11. Chromatogram of the total ion current for the LC−MS separation on the column packed with blank polymer BP1.

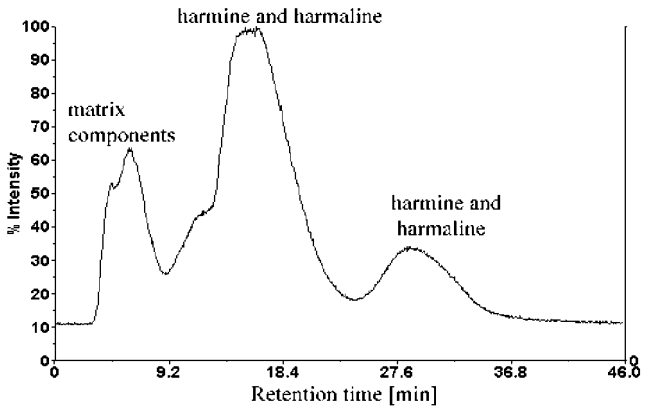

Figure 12. Chromatogram of the total ion current for the LC−MS separation on the column packed with blank polymer BP2.

harman and the carboxy groups on the polymers. With a high content of water in acetonile, the P1 column exhibited enhanced retention for harman, but the P2 column did not. This suggested that the network of P1 was more hydrophobic than that of P2. Consequently the low-polar interferences in the crude herb solution were inclined to nonspecifically adsorb on P1 and led to the poorer separation performance of the P1 column.

The MIP-LC−MS separation results are shown in Figures 7 to 15. It was reasonable that the sample size injected on the column P2 was less than that on the P1 column, because P2 possessed a smaller binding capacity than P1. When the sample size injected on the P2 column was the same as that on the P1 column, the fractions of harmine and harmaline were eluted out close to the fractions of the matrix components, which made the LC−MS separation result on the P2 column poor. It can be seen

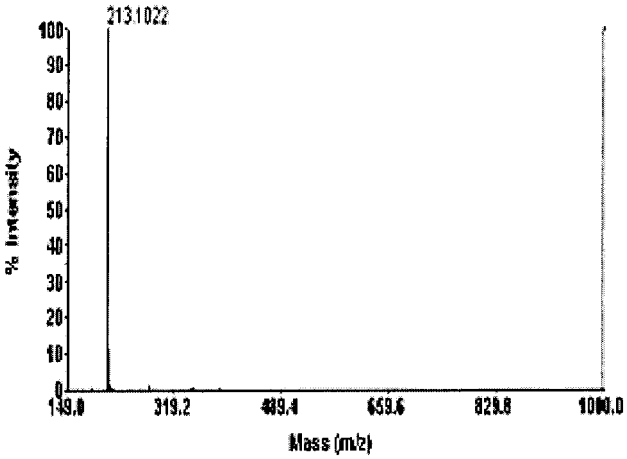

Figure 13. Mass spectra of the extracted ion corresponding to harmine.

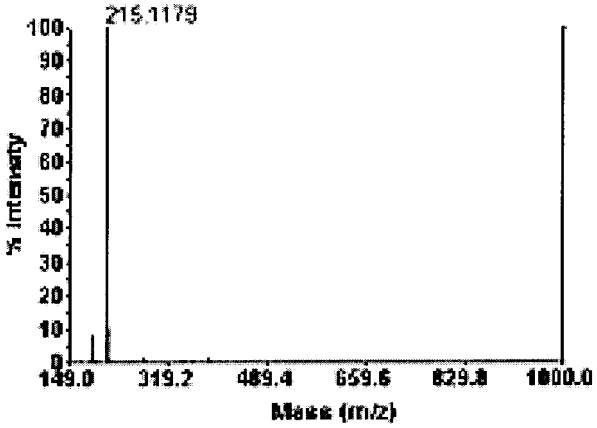

Figure 14. Mass spectra of the extracted ion corresponding to harmaline.

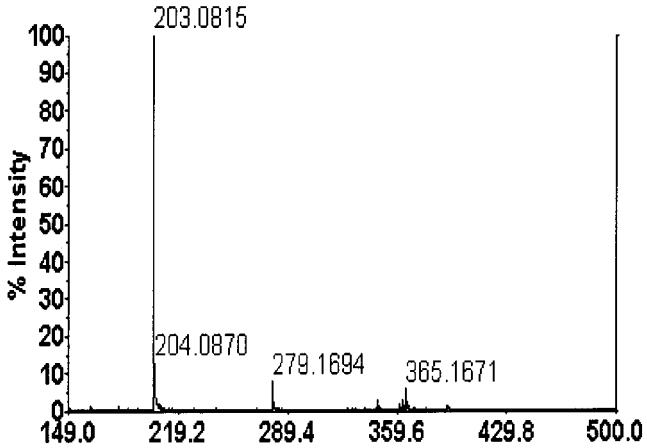

Figure 15. Mass spectra of the extracted ion corresponding to vasicinone.

in Figure 7 or Figure 9 that because the MIPs nonspecifically adsorbed matrix components from the herb extract, the capacity factors of harmine and harmaline became smaller than they should be when injected independently. However, it was feasible for harmine and harmaline, the antitumor components, to be selectively extracted from the herb extract by P1 or P2. It could also be observed from Figure 7 or Figure 9 that harmine and harmaline were eluted out much later as a result of their high retention on

the MIPs, whereas nonspecific matrix components in the herb extract were coeluted out in less than 20 min. Harmine was partly separated from harmaline on the P1 column (Figure 8), whereas the two compounds were not resolved on the P2 column (Figure 10). This was reasonable, considering P1 possessed higher affinity and specificity for the targets than did P2. Moreover, specificity of the MIPs in the LC−MS test could be further confirmed from the extracted ion chromatograms (Figures 8 and 10) and their corresponding mass spectra (Figures 13−15). It can be observed that vasicinone (Figure 15), another nitrogen-based compound in the extract of *P. nigellastrum* seeds,[33] was eluted out and identified as a matrix component in the LC−MS test. However, vasicinone (m/z 203) was not detected when we analyzed the herb extract solution by the off-line ESI-MS due to matrix interferences, which demonstrated the potential usefulness of on-line LC−MS. In contrast, the BP columns could not achieve the above separation results. It can be seen from Figure 11 that harman and harmaline were not extracted out by BP1, because harmine and harmaline were coeluted out with the matrix compounds. Similarly, the antitumor components of harmine and harmaline were poorly separated from the matrix components by BP2 (Figure 12). The splitting of the peak of harmine and harmline on BP2 (Figure 12) might be associated with its low column efficiency, since polymer particles we used were in irregular shapes. Conclusively, better separation could only be obtained by the imprinted phases.

CONCLUSIONS

Polymers with affinity and selectivity for the antitumor compounds£ harmine and harmaline were synthesized using their structural analogue harman as the template. The antitumor components, harmine and harmaline, were efficiently separated and identified from the herb extract by combining the anti-harman MIP columns with a MS detector, which demonstrated that it was possible to obtain compounds of specific pharmacophoric features from traditional Chinese herbs by using target analogue receptors as the adsorbents. As we know, in the present imprinting protocols, obtaining imprints of natural compounds is often problematic, because they are expensive or difficult to obtain or they are poorly soluble in weak polar organic solvents. Our approach is expected to be helpful for the further extraction of certain pharmacophoric compounds from herb extracts by the molecular imprinting technique.

MIPs in different porogens were prepared and characterized by the chromatographic mode, and the binding capacities of the MIPs were determined by frontal chromatography. Afterward, performances of the MIPs in separating the herb extract were investigated by coupling the MIP columns with an APCI-TOFMS detector. It was demonstrated that the porogens used at the imprinting step affected the selectivity and affinity of the MIPs as well as their separation performances when they were used as HPLC solid phases to directly separate samples of herb extract. Indeed, the MIP prepared using a porogen of less hydrogen bonding capacity possessed a higher affinity and selectivity for the targets. However, because of its hydrophobic polymeric matrix, the separation was seriously disturbed in the LC−MS test as a result of its nonspecific adsorption of the low polar matrix components from the herb extract. Intriguingly, the MIPs were still stable and reproducible after they were subjected to the crude extract, and the imprinted columns could selectively separate the antitumor components in the herb extract after the sample was simply pretreated.

Above all, by using the MIP-LC−MS system, not only the antitumor components, harmine and harmaline, can be efficiently separated and confirmed from the herb extract in its native form, but also another nitrogen-based compound, vasicinone, was recognized and identified in such a LC−MS system. This further indicated the potential usefulness of combining columns of MIPs with the detection of MS for the discovery of lead compounds from medicinal herbs.

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