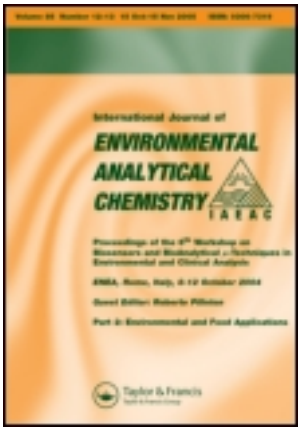

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Development of a broad selective molecularly imprinted polymers-based solid phase extraction of contraceptive drug levonorgestrel from water samples

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Development of a broad selective molecularly imprinted polymers-based solid phase extraction of contraceptive drug levonorgestrel from water samples

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A broad selective molecularly imprinted polymers-based solid phase extraction (MISPE) for levonorgestrel (LNG) from water samples was developed. Using LNG as a template molecule, acrylamide (AA) as functional monomer, ethylene glycol dimethacrylate (EGDMA) as linking agent and bulk polymerisation as a synthetic method, the molecularly imprinted polymers (MIPs) were synthesised and characterised. The MIPs displayed a high specific rebinding for LNG with the imprinting factor of 3.71. The Scatchard analysis showed that there was at least one class of binding site for LNG formed in the MIPs with the dissociation constant of $8.046\,\mu g\,mL^{-1}$. The results of selectivity testing indicated that the MIPs also exhibited high cross-reactivity with structurally related compounds (estrone, methylprednisolone and ethinyl estradiol), but no recognition with non-structurally related compound (indomethacin), suggesting that the MIPs could be used as a broad recognition absorbent. MISPE column was prepared by packing MIPs particles into a common SPE cartridge. The MISPE extraction conditions including loading, washing and eluting solutions were carefully optimised. Water samples spiked with LNG were extracted by MISPE column and detected by high-performance liquid chromatography. The recoveries were found to be $79.97 \sim 132.79\%$ with relative standard deviations (RSD) of $1.92 \sim 10.43\%$, indicating the feasibility of the prepared MIPs for LNG extraction.

Keywords: molecularly imprinted polymers (MIPs); levonorgestrel; solid phase extraction (SPE); water samples

1. Introduction

Levonorgestrel (LNG, Figure 1), one kind of synthetic female contraceptive drug [1], has been widely used in pregnancy prevention in humans based on its relatively low price and good efficacy. However, during recent years, it has been reported that LNG is genotoxic to human peripheral blood lymphocytes *in vitro* and also has deleterious reproductive effects including the turbulence of catamenia, increasing rate of galactophore cancer, etc. [2–4]. LNG and other steroids are normally excreted from the human body, entering the receiving water through sewage effluent. Long-time existence of such drugs at trace concentration in aquatic systems may disrupt the endocrines of aquatic animals. LNG and

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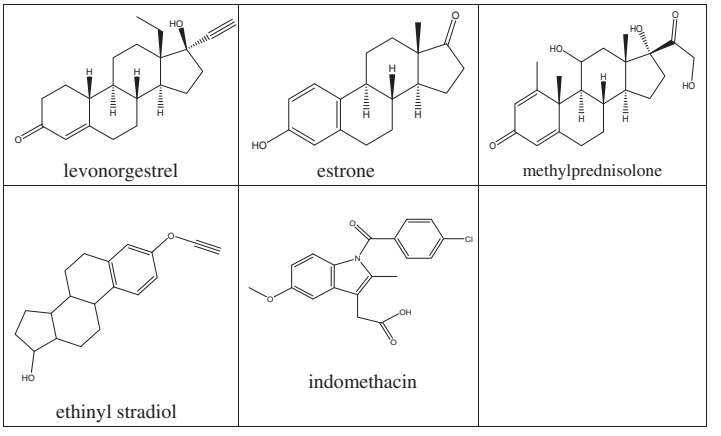

Figure 1. The molecular structures of levonorgestrel, estrone, methylprednisolone, ethinyl estradiol and indomethacin.

other steroids are stable in the environment, so they may accumulate in human bodies through the food-chain and cause a potentially adverse effect on humans.

LNG is usually determined by instrumental analysis such as gas chromatography-mass spectrometry (GC-MS) [5], GC-MS/MS [6], liquid chromatography-diode array detector (LC-DAD) [4], LC-DAD-MS [7,8], LC-MS/MS [9] and LC-MS/MS with electrospray ionisation (ESI) [10] or with atmospheric pressure chemical ionisation (APCI) [11]. In most cases, these chromatographic methods could detect trace levels of target analytes when coupled with an extensive pre-treatment step such as solid phase extraction (SPE). However, current SPE is based on physicochemical retention on a functionalised surface which captures not only the target analyte but also other matrix components [12,13]. The drawbacks of routine SPE techniques are their low selectivity towards the particular target analyte. New efficient cleanup techniques employing sorbents with a high selectivity for analyte are indispensable.

Molecularly imprinted polymers (MIPs) are synthetic cross-linked polymers that possess tailor-made cavities suited for a given target template [14,15]. The MIPs are produced by polymerisation of a solution containing target template molecules and functional monomers after adding cross-linker and initiator. Before polymerisation, the functional monomer interacts with the template by non-covalent forces such as hydrogen, hydrophobic or ionic bonds. After polymerisation and removal of template molecule, the final materials retain the specific orientation of functional groups within the cavity which is complementary to the template molecule in size, shape and functionality. MIPs offer a high affinity for the analyte compared with silica-based and other polymeric sorbents. On the other hand, MIPs are easy and rapid to prepare, and can resist harsh conditions such as high concentration of organic solvents, elevated temperatures and pressures, strong acids and bases, etc. MIPs have been exploited as separation materials [16], chemical sensors [17], reaction catalysts [18], enzyme mimics [19], and in particular, as SPE adsorbents (MISPE) [20–26].

To our knowledge, there was only one published paper concerning the molecular imprinting using LNG as a template. Using methacrylic acid (MAA) as a functional monomer, and ethylene glycoldimethacrylate (EGDMA) as a linking agent,

Khorrami, and Mehrseresht reported the preparation of MIPs by free radical polymerisation in chloroform [27]. The MIPs were characterised in terms of binding property and selectivity. The equilibrium rebinding was tested in LNG standard concentrations of 15–95 μmol L^{-1} (e.g. 4.7–29.7 μg mL^{-1}). The equilibrium dissociation constant was 55 μmol L^{-1} (e.g. 19.2 μg mL^{-1}) and the LNG imprinted polymer was applied for selective solid phase extraction of LNG from human serum. As LNG has no ionisable function (acidic and basic), a stronger specific binding MIP may be prepared when using LNG as template molecule and acrylamide (AA) as functional monomer, owing to the strong hydrogen bonds between the amino groups in AA and the carbonyl groups in LNG.

The aim of this study is to synthesise, characterise and apply a MISPE technique for LNG extraction from water samples. Using LNG as template molecule, acrylamide (AA) as functional monomer, ethylene glycol dimethacrylate (EGDMA) as linking agent, the MIPs were prepared by bulk polymerisation and characterised with rebinding experiment. The MISPE column was prepared by filling the MIPs particles into a common SPE column and the experimental conditions such as loading, washing and eluting solutions were carefully optimised. Upon optimal conditions, water samples were extracted by MISPE column and analysed by HPLC.

2. Experimental

2.1 *Chemicals and apparatus*

Levonorgestrel (LNG), acrylamide (AA) and ethylene glycol dimethacrylate (EGDMA) were purchased from Fluka (Buchs, Switzerland). EGDMA was redistilled under vacuum before use. 2, 2-azoisobutyronitrile (AIBN), methanol, acetone, dimethyl sulfoxide (DMSO), trifluoroacetic acid and acetonitrile were from Ke-long Chemical Company (Chengdu, China). Estrone and ethinyl estradiol were from Sigma (St. Louis, MO). Methylprednisolone was from Zhejiang Xianjun Pharmaceutical Co, Ltd (China) and indomethacin was from Shanghai No. 10 pharmaceutical factory (Shanghai, China). Methanol and acetonitrile (ACN) of HPLC grade are purchased from Fisher Scientific (Pittsburgh, PA, USA). All reagents mentioned above are of analytical grade. LNG stock solution (100 μg mL^{-1}) was prepared with acetonitrile and stored at 4°C.

Ultraviolet visible spectrophotometer (TU-1901) was from Beijing Purkinje General Instrument Co. Ltd. (Beijing, China). Electronic balance (BS 124S) was from Sartorius Com. (Gottingen, Germany). The Deionized-RO water supply system (DZG-303A) was from AK Company (Joint Company between Chengdu and Taiwan, Chengdu, China). Constant temperature oscillator (SHZ-88) was from Jiangsu Experimental Instruments Factory (Taicang, Chin). C_{18} SPE columns were from Drace Davision Discovery Sciences (Deerfield, IL, USA). HPLC system was from Alltech Associates, Inc. (Deerfield, IL, USA).

2.2 *Preparation of MIPs*

MIPs were prepared as follows. Briefly, 2.0 mmol of LNG and 8.0 mmol of AA were dissolved in 8.0 mL DMSO in a 50 mL glass flask. The mixture was placed into the water bathing at 30°C for 6 h, then 40.0 mmol of EGDMA and 0.48 mmol initiator AIBN were added. The flask was degassed in an ultrasonic bath for 5 min, then purged with nitrogen for 10 min and pumped vacuum for 10 min. The flask was sealed and the polymerisation

was carried out in a thermostatic water bath at 60°C for 24 h. After polymerisation, the polymers were dried under infrared heat lamp, then mechanically ground in a mortar and sieved to select the particles between 35 μm and 75 μm in size. Fine particles were removed by three cycles of sedimentation in acetone. The selected particles were placed in Soxhlet apparatus and washed with methanol/trifluoroacetic acid (9 : 1, v/v) for 48 h. To remove the imprinted template molecules from the MIPs particles as much as possible, the polymer particles were packed in a glass column and washed intensively with methanol at flow rate of 1 mL min^{-1}. The eluate was monitored by UV spectrophotometer at 242 nm until no residual template was detected. Finally, the polymers were dried and stored in a desiccator at ambient temperature. The non-imprinted polymers (NIPs) were prepared in the same way without the addition of the template molecule.

2.3 *Equilibrium rebinding test of MIPs and Scatchard analysis*

Equilibrium rebinding test was carried out by adding 30 mg MIPs or NIPs in a vial containing 3.00 mL of LNG standard solution varying in $10 \sim 60\ \mu g\ mL^{-1}$ prepared with acetonitrile. The solutions suspended with MIPs were incubated at 25°C by continuously shaking for 18 h. The suspension was filtrated with a 0.45 μm filter and detected by UV spectrophotometer at 242 nm. The amount of LNG bound on the polymers was obtained by subtracting the free concentration from initial LNG.

The Scatchard plot was constructed according to the equation: $(B/[F]) = -(B/K_d) + (B_{max}/K_d)$, where B is the amount of LNG bound on MIPs at equilibrium; $[F]$ is the free analyte concentration at equilibrium; K_d is the dissociation constant and B_{max} is the apparent maximum binding amount. The values of K_d and the B_{max} can be calculated from the slope and intercept of the linear line plotted in $B/[F]$ versus B.

2.4 *Selectivity of MIPs*

Besides LNG, three structurally related compounds (estrone, methylprednisolone, ethinyl estradiol) and another non-structurally related compound (indomethacin) were employed to test the selectivity of the MIPs (the molecular structures of estrone, methylprednisolone, ethinyl estradiol and indomethacin are shown in Figure 1). Three millilitres of testing compounds were dissolved in acetonitrile at a concentration of $20\ \mu g\ mL^{-1}$ and added to 30 mg of MIPs or NIPs. The mixture was incubated at 25°C for 18 h under slightly shaking, then the mixture solution was filtrated with 0.45 μm filter and the supernatant was determined with UV spectrophotometer at corresponding maximum absorption wavelength (estrone, methylprednisolone and ethinyl estradiol: 243 nm; indomethacin: 218 nm). The amount of testing compounds adsorbed to the polymers was calculated by subtracting the concentration of free compound from the initial concentration.

2.5 *Preparation of MISPE cartridge and optimization of extraction conditions*

MISPE (or NISPE) cartridge was prepared by packing 100 mg of MIPs (or NIPs) into an empty SPE cartridge (4 mL) with polyethylene frits placed at the top and the bottom. Before extracting water samples, the cartridge was consecutively conditioned with 3 mL of acetonitrile, 3 mL of methanol and 1 mL of deionised water.

The extraction conditions such as loading, washing and eluting solution were important factors affecting extraction efficiency. In this study, as the aim was to extract LNG from water sample, therefore pure water was firstly tested whether it would be used as a loading solution. 1 mL of 1.0 μg mL^{-1} of LNG standard solution prepared in pure water was loaded onto the MISPE column at the flow rate of 0.5 mL min^{-1}. All effluents from loading step was collected and detected by UV spectrophotometer at 242 nm. During the process of loading sample, all analyte should be selectively absorbed on the MIPs, but some extent of non-specific binding might be unavoidable because of native adsorption ability of fine polymer particles. To remove non-specific binding sufficiently and keep the specific binding remained, several washing solutions (e.g. 4 ml pure water containing 10%, 20%, 40%, 50%, 60% and 70% of methanol; and 4 ml of 60% methanol with the pH values of 2, 3, 4, 5, 6, 7, 8) were tested to find optimal washing condition. After washing step, all specifically bound analyte should be eluted from the column. In this study, three eluting solutions (e.g. pure methanol, methanol/trifluoroacetic (9 : 1, v/v) and pure acetonitrile) were tested to find the optimal eluting solution.

2.6 *Spiking experiment and analysis of real water samples*

Under optimal extraction conditions, the MISPE column was characterised in terms of precision and accuracy, which can be achieved by spiking experiment. River water samples collected from Funan river (Chengdu) were spiked with LNG at the concentration of 0.5, 1.0, 5.0 and 10 ng mL^{-1}. 40 mL water samples spiked with LNG at different concentration was separately loaded to MISPE column (The amount of LNG loaded on the column was 20, 40, 200 and 400 ng, respectively). All fractions from washing and eluting steps were collected and detected by HPLC. The extraction procedures were repeated three times.

Three other water samples (e.g. a river water collected from the Jiangan river, Chengdu; an influent and an effluent collected from the wastewater treatment plant, Chengdu) without LNG spiking were extracted with MISPE column. After filtrating with 0.45 μm filter to remove sediments or particles, 40 mL of water samples were loaded onto MISPE column, and the fractions from washing and eluting steps were collected and detected by HPLC.

2.7 *HPLC detection*

A HPLC system (Alltech, USA) with C_{18} column (Alltech, USA, Allitima^{TM}: 4.6 mm × 250 mm, 5 μm) was employed for quantitative detection of LNG. A 20 μL sample loop was used and the detection was realised with a UV-vis 201 detector integrated into the HPLC. The mobile phases and flow rate of HPLC for LNG was acetonitrile/water (60 : 40, v/v) at 1.0 mL min^{-1}. The UV detection wavelength was set at 242 nm. The HPLC standard curve for LNG was constructed at the concentrations of 0, 0.05, 0.125, 0.25, 0.5, 1, 2, 4 μg mL^{-1}.

3. Results and discussion

3.1 *Preparation of MIPs*

For non-covalent molecular imprinting, the specific molecular recognition is based on the complementary interactions between a template and functional monomers. After the

template molecule is decided, for the preparation of a MIP, the choice of appropriate functional monomer and porogen is needed. It is well known that both acrylamide (AA) and methacrylic acid (MAA) are the most functional monomers used in the preparation of MIPs. In this study, we synthesised the MIPs of both AA and MAA as functional monomers and it was found that the imprinting efficiency of MIP using AA as functional monomer was better than that using MAA as functional monomer (the results were not presented herein). Therefore, AA was selected as a functional monomer, instead of MAA used by Khorrami and Mehrseresht [27]. The stronger non-covalent interactions may be attributed to the hydrogen bonds between the amino groups in AA and the carbonyl groups in LNG. On the other hand, we prepared two types of MIPs using DMSO and chloroform as porogens. It was observed that the imprinting efficiency of the MIP using DMSO as a porogen was higher than that using chloroform as a porogen (the results were not presented herein). It was also noticed that the solubility of LNG in acetonitrile was lower than that in DMSO (or chloroform). Therefore DMSO was chosen as a porogen in this study. In addition, to make the embedded template molecules removed as much as possible, the MIPs particles were intensively extracted with Soxhlet for 48 h; after being packed in a glass column, they were also intensively washed with methanol at flow rate of $1\,mL\,min^{-1}$ until no residual template was detected in the eluate. As a control, NIPs were treated simultaneously with the same protocols as those for MIPs.

3.2 *Equilibrium rebinding study and Scatchard analysis*

In most cases, the equilibrium rebinding study was carried out in the same organic solvent as that in the polymerisation process used as the porogen. It is supposed that, in that condition, the rebinding capacity of the MIPs' particles for template molecule will be the highest [28]. In this study, the equilibrium rebinding of the MIP for LNG was tested in acetonitrile instead of the porogen (DMSO) because the cut-off absorption wavelength of DMSO was at 268 nm, which would interfere with the UV detection of the analyte.

To investigate the recognition properties of the MIP for the analyte, different initial concentration of LNG of $10 \sim 60\,\mu g\,mL^{-1}$ were applied in equilibrium rebinding experiments. The resulting binding isotherms of MIP and NIP for LNG are shown in Figure 2. It was clear from this that the amount of LNG specifically adsorbed on the MIP increased rapidly with the initial concentration of LNG from $10\,\mu g\,mL^{-1}$ to $40\,\mu g\,mL^{-1}$ and saturated at an LNG concentration of $>40\,\mu g\,mL^{-1}$; while the amount of LNG non-specifically adsorbed on the NIP increased slowly with the initial concentration of LNG from $10\,\mu g\,mL^{-1}$ to $25\,\mu g\,mL^{-1}$ and saturated at an LNG concentration of $>25\,\mu g\,mL^{-1}$. The imprinting factor which was defined as the ratio of the amount of template molecule adsorbed on MIP to that on NIP was 3.71, indicating high specific recognition of the prepared MIP for the analyte.

The rebinding data were further processed using Scatchard analysis. Scatchard analysis is an approximate binding isotherm model, but it is commonly used in MIPs characterization [13,29]. The results are illustrated in Figure 3. It was clear that the Scatchard plot was a single straight line. The linear regression equation was: $B/[F] = -0.124B + 170.924$ ($R^2 = 0.929$), suggesting that at least one class of binding site for LNG was formed in the MIP [13,29]. From the slope and intercept of the line, the values of K_d and B_{max} were found to be $8.046\,\mu g\,mL^{-1}$ and $1375.199\,\mu g\,g^{-1}$, respectively.

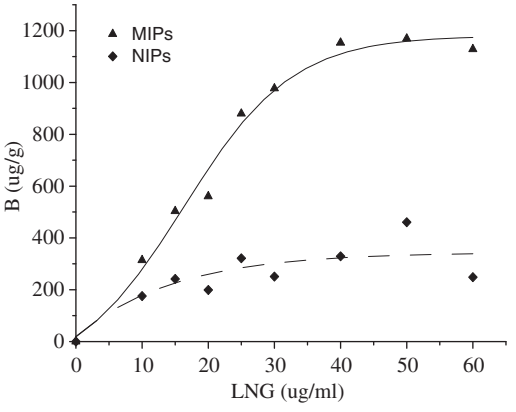

Figure 2. Binding isotherms of LNG on MIPs and NIPs in acetonitrile. LNG concentration: $10 \sim 60\,\mu g\,mL^{-1}$; volume of LNG solution: 3.0 mL; binding time: 18 h.

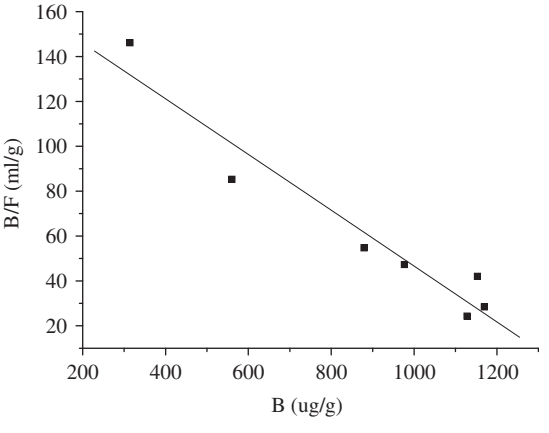

Figure 3. Scatchard plot of the rebinding of LNG on MIPs. B is the amount of LNG bound to the polymer; [F] is the concentration of free LNG at equilibrium.

3.3 *Selectivity of MIPs*

Besides LNG, estrone, methylprednisolone, ethinyl estradiol and indomethacin (Figure 1) were employed to test the selectivity of the MIPs. Because they have the similar structure frame, estrone, methylprednisolone and ethinyl estradiol are structurally related compounds with LNG, while the molecular structure of indomethacin was different to that of LNG. The rebinding situations of LNG, estrone, methylprednisolone, ethinyl estradiol and indomethacin on the MIPs and NIPs are illustrated in Figure 4. Obviously, the binding of LNG, estrone, methylprednisolone and ethinyl estradiol on MIPs was higher than that on NIPs, while the binding of indomethacin on MIPs was very close to that on NIPs. The cross-reactivity (CR) values of MIPs with LNG, estrone, methylprednisolone, ethinyl estradiol and indomethacin [CR(%) = (the difference of amount of tested

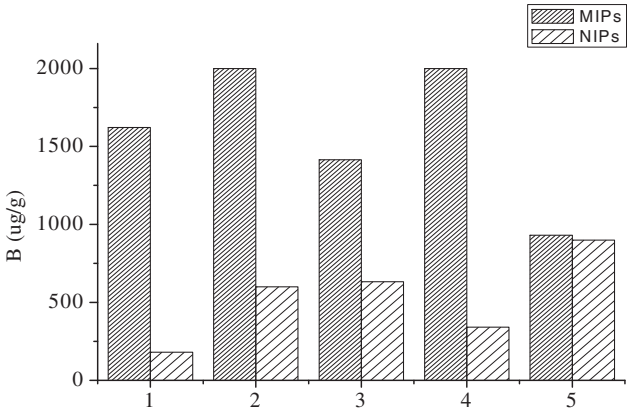

Figure 4. The binding amount of LNG, estrone, methylprednisolone, ethinyl estradiol and indomethacin on MIPs. MIPs amount: 30 mg; binding time: 18 h; binding medium: acetonitrile; compounds concentration: 20 μg mL^{-1}. 1-LNG; 2-estrone; 3-methylprednisolone; 4-ethinyl estradiol; 5-indomethacin.

compound bound on MIP and NIP)/(the difference of amount of LNG bound on MIP and NIP) × 100%] were 100.0%, 97.2%, 54.6%, 115.4%, 2.1%, respectively, which demonstrated that the prepared MIPs not only displayed highly specific recognition with LNG, but also exhibited high rebinding with structurally relative compounds. The high cross-reactivity of the MIPs with estrone, methylprednisolone and ethinyl estradiol may be mainly attributed to the complementary of the cavity of MIPs with the spatial structure of the tested compounds. It can be seen from Figure 1 that the molecular structures of estrone and ethinyl estradiol are very similar to that of LNG, and more important, the spatial size of estrone and ethinyl estradiol is a little smaller than that of LNG, which allowed the estrone and ethinyl estradiol to match the size and shape of MIPs cavity sufficiently. Although the spatial diameter of the methylprednisolone is somewhat different from that of LNG, owing to the swelling effect of solvent [30], it is possible that methylprednisolone can enter the cavity of the MIPs without difficulty. Therefore, the MIPs might be used as a broad selective extraction adsorbent. As the structure of the indomethacin was quite different from that of LNG, the MIPs almost did not recognise indomethacin.

3.4 *Optimisation of MISPE conditions*

The extraction conditions such as loading, washing and eluting solution should be carefully optimised. In this study, the aim was to extract LNG from a water sample, so pure water was first tested to determine whether it could be used as loading solution. 1 mL of 1.0 μg mL^{-1} of LNG standard solution prepared in pure water (e.g. 1.0 μg LNG) was loaded onto the MISPE column at the flow rate of 0.5 mL min^{-1}. During loading, all effluents from the MISPE columns were collected and detected. It was found that there was no detectable LNG in the effluents, which indicated that when pure water was used as a loading solution, all of the analyte was completely bound on the MIPs.

To find out the appropriate washing solution to wash out non-specific binding and to remain specific adsorption, several washing solutions (e.g. 4 ml pure water containing

10%, 20%, 40%, 50%, 60% and 70% of methanol; and 4 ml of 60% methanol with the pH values of 2, 3, 4, 5, 6, 7, 8) were tested. When a total 1.0 μg of LNG in pure water was respectively loaded onto the NISPE and MISPE columns, the columns were separately washed with 4 ml pure water containing 10%, 20%, 40%, 50%, 60% and 70% of methanol. It was found that with the percentage of methanol increasing, the amount of non-specific binding removed from NISPE column was gradually increased, and at 60% of methanol, the non-specific binding removed from the NISPE column reached a maximum value (85%). While for MISPE column, at 60% of methanol, 88% of specific binding remained on the column. To further improve washing efficiency, 4 ml of 60% methanol with the pH values of 2, 3, 4, 5, 6, 7, 8 was tested. It was found that at 60% methanol with the pH values of 4, more than 92% of nonspecific binding was removed from the NISPE column and 94% of specific binding remained on MISPE column. Therefore, pure water containing 60% of methanol with pH value of 4 was selected as washing solution.

After the washing step, all analyte specifically bound on the column should be eluted from MISPE column. Three eluting solutions (e.g. pure methanol, methanol/trifluoroacetic (9 : 1, v/v) and pure acetonitrile) were tested. It was found that at pure acetonitrile as eluting solution, all analyte was removed from MISPE column. Therefore, pure acetonitrile was selected as an eluting solution.

3.5 *Spiking experiment and real sample analysis*

Under optimal extraction conditions, to characterise MISPE column in terms of precision and accuracy, a spiking experiment was performed. River water samples collected from Funan river (Chengdu) were spiked with LNG at concentrations of 0.5, 1.0, 5.0 and 10 ng mL^{-1}. 40 mL water samples spiked with LNG at different concentration was separately loaded to MISPE column (LNG loaded was 20, 40, 200 and 400 ng, respectively). After loading, the column was washed with 4 mL pure water containing 60% methanol with the pH value of 4, then eluted with 3 mL of acetonitrile. All washing and eluting fractions were collected and then dried under nitrogen at 40°C. The residues were dissolved with a 0.5 mL mobile phase (acetonitrile/water, 60/40, v/v) with 80 extraction folds (e.g. 40/0.5). Then, 20 μl of solution was injected into the HPLC column. The UV detection wavelength was set at 242 nm. The extraction procedures were repeated three times. The results of the spiking experiment are summarised in Table 1. It can be seen from Table 1 that the values of the relatively standard deviation (RSD) were in the range of 1.92 ~ 9.68%, while the recoveries were within 79.97 ~ 132.79%, indicating good precision and accuracy of the MISPE column for extraction of LNG from water samples.

Figure 5 was the chromatogram of LNG after 80 folds' extraction by MISPE column where 40 mL of the Funan river water spiked with LNG at concentration 0.5 ng mL^{-1} was loaded.

Three other water samples (e.g. Jiangan river water sample, an influent and an effluent from wastewater treatment plant) without LNG spiking were extracted with MISPE column. Filtrating with 0.45 μm filter to remove sediments or particles, 40 mL of water samples were loaded to the MISPE column. After washing and eluting the column, the washing and eluting fractions were collected and dried. The residues were reconstituted by 0.5 mL mobile phase with the 80 extraction folds. Then, 20 μl of solution was injected into

Table 1. Precision and accuracy of MISPE column for the extraction of LNG from 40 mL spiked water samples.

LNG spiked (ng)	LNG detected (ng)* Mean ± SD ($n = 3$)	RSD (%)	Recovery (%)
20	23.11 ± 5.84	9.68	115.57
40	48.20 ± 5.75	5.21	120.49
200	265.58 ± 4.74	1.92	132.79
400	319.89 ± 7.63	3.28	79.97

*There was no detectable LNG in blank Funan river water after 80 extraction folds.

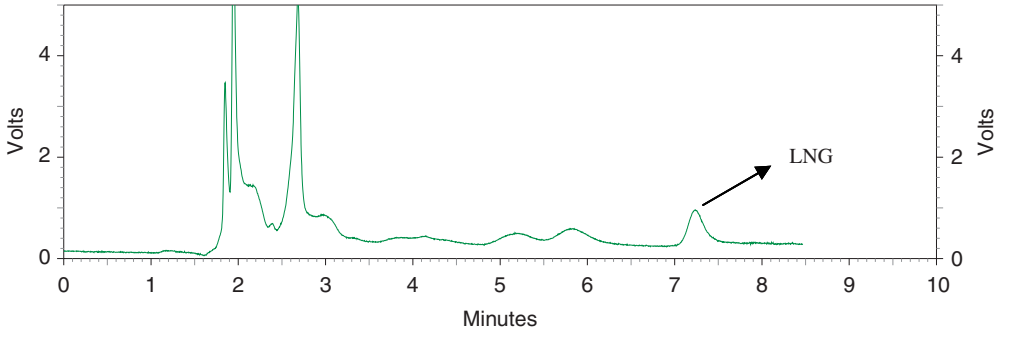

Figure 5. Chromatogram of LNG detected by HPLC. 40 mL of Funan river water spiked with LNG at concentration 5.0 ng mL^{-1} was loaded onto MISPE column and detected by HPLC.

HPLC column for LNG detection. It was found that there was no detectable LNG for these three water samples.

The reusability of the MISPE column should be addressed. After one cycle of extraction, the column should be consecutively washed with 3 mL acetonitrile, 3 mL methanol and 3 mL deionised water. The MISPE column can be reused more than 30 times without loss of extraction capability.

In conclusion, using LNG as template molecule, the MIPs were successfully prepared by bulk polymerisation and characterised by rebinding experiment. The MIPs not only displayed high specific recognition with LNG, but also showed high cross-reactivity values with structurally related compounds, suggesting that MIPs could be used as broad specific adsorbents in solid phase extraction. The MISPE column was prepared by packing the MIP particles into a common SPE cartridge and the extraction conditions during the processes of loading, washing and eluting were optimised. By using pure water containing 60% of methanol (pH 4) as washing solution, most non-specific binding was eliminated. Good precision and accuracy of the MISPE column for LNG in spiked water samples demonstrated the feasibility of the prepared MIP for LNG extraction.

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References

[1] H.B. Croxatto, S. Diaz, A.M. Salvatierra, P. Morales, C. Ebenserger, and A. Brandeis, Contraception **36**, 193 (1987).
[2] M.E. Ahmed, G.A. Shadab, M.A. Azfer, and M. Afzal, Mutat. Res-gen. tox. en. **494**, 13 (2001).
[3] S.L. Camp, D.S. Wilkerson, and T.R. Raine, Contraception **68**, 309 (2003).
[4] T. Backman, Drug Safety **27**, 1185 (2004).
[5] H.M. Kuch and K. Ballschmiter, Fresenius J. Anal. Chem. **366**, 392 (2000).
[6] C.H. Huang and D.L. Sedlak, Environ. Toxicol. Chem. **20**, 133 (2001).
[7] M.H. Choi, K.R. Kim, and B.C. Chung, J. Chromatogr. A **892**, 391 (2000).
[8] M.J. Alda and D. Barceló, J. Chromatogr. A **938**, 145 (2001).
[9] C.W. Cui, S.L. Ji, and H.Y. Ren, Environ. Monit. Assess. **121**, 409 (2006).
[10] C. Baronti, R. Curini, R.G. D'Ascenzo, A.D. Corcia, A. Gentili, and R. Samperi, Environ. Sci. Technol. **34**, 5059 (2000).
[11] P. Adler, T. Steger-Hartmann, and W. Kalbfus, Acta Hydroch. Hydrob. **29**, 227 (2001).
[12] C. Baggiani, P. Baravalle, G. Giraudi, and C. Tozzi, J. Chromatogr A **1141**, 158 (2007).
[13] Q.Z. Zhu, P. Degelmann, R. Niessner, and D. Knopp, Environ. Sci. Technol. **36**, 5411 (2002).
[14] K.J. Shea and D.Y. Sasaki, J. Am. Chem. Soc. **113**, 4109 (1991).
[15] G. Wulff and S. Schauhoff, J. Org. Chem. **56**, 395 (1991).
[16] G.L. Yang, H.Y. Liu, and M.M. Wang, React. Funct. Polym. **66**, 579 (2006).
[17] S.A. Piletsky and E.V. Piletskaya, Biosens. Bioelectron **10**, 959 (1995).
[18] C. Alexander, L. Davidson, and W. Hayes, Tetrahedron **59**, 2025 (2003).
[19] M. Klaus, Y. Yihua, A. Jens, and Y. Lei, J. Am. Chem. Soc. **123**, 12420 (2001).
[20] B. Sellergren, Anal. Chem. **66**, 1578 (1994).
[21] H. Jamil, B. Man, and O. Philip, J. Chromatogr. A **1112**, 104 (2006).
[22] J.M. Lin, T. Nakagama, and K. Uchiyama, Chromatographia **43**, 585 (1996).
[23] C. Baggiani, L. Anfossi, and C. Giovannoli, Anal. Chim. Acta **591**, 29 (2007).
[24] S. Hu, S. Wang, and X. He, Analyst **128**, 1485 (2003).
[25] F.G. Tamayo, E. Turiel, and A. Martín-Esteban, J. Chromatogr. A **1152**, 32 (2007).
[26] E. Turiel, A. Martín-Esteban, and J.L. Tadeo, J. Chromatogr. A **1172**, 97 (2007).
[27] A. Rajabi Khorrami and S. Mehrseresht, J.Chromatogr. B **867**, 264 (2008).
[28] K. Yoshizako, K. Hosoya, and Y. Iwakoshi, Anal. Chem. **70**, 386 (1998).
[29] T. Yang, S. Wei, Y.H. Li, Y. Li, and A.P. Deng, Anal. Bioanal. Chem. **391**, 2905 (2008).
[30] K. Yoshizako, K. Hosoya, Y. Iwakoshi, K. Kimata, and N. Tanaka, Anal. Chem. **70**, 386 (1998).