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Dufulin is a newly developed α–aminophosphonates antiviral agent and displays a high antiviral activity towards tobacco mosaic disease. During the environmental assessment of Dufulin, the traditional separation and purification techniques have low–separation efficiency, with a large quantity of organic solvents consumed, tedious operations, and potential impurity introduction. Therefore, developing a highly sensitive, reliable and selective method to determine Dufulin at trace level is necessary. In this study, a novel procedure was developed to synthesize Dufulin–imprinted silica gel sorbent with a surface molecular imprinting technique. The new method could allow us to efficiently separate and purify Dufulin from environmental samples.

**Title:** Preparation of Dufulin imprinted polymer on surface of silica gel and its application as solid–phase extraction sorbent

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**Abstract**

A new molecularly imprinted polymer (MIP) based on silica–gel surface was developed using Dufulin (Duf) as a template, methacrylic acid (MAA) as a functional monomer, ethyleneglycol dimethacrylate (EGDMA) as a crosslinker, and azodiisobutyronitrile (AIBN) as an initiator. The synthetic samples were characterized by the techniques of fourier transmission infrared spectrometry (FT–IR) and scanning electron microscope (SEM). Batch experiments were performed to evaluate adsorption isotherms, adsorption kinetics and selective recognition of the MIP. Binding experiments demonstrated that the MIP had a good adsorption capacity, fast mass transfer rate and high recognition selectivity to Dufulin. When the MIP was used as solid–phase extraction (SPE) materials, the recoveries of Dufulin for spiked water, soil and wheat samples were 88.98–102.16%, 85.31–99.57% and 87.84–100.19%, along with LOD of 0.0008 mg $L^{-1}$, 0.010 mg $kg^{-1}$ and 0.023 mg $kg^{-1}$, respectively. Compared with direct determination of HPLC without MIP-SPE, the highly selective separation and enrichment of Dufulin from the complex environmental media can be achieved by the newly developed molecular imprinting at the surface of silica gel.

**Keyword:** Dufulin; silica gel; molecularly imprinted polymers; surface imprinting technique; solid–phase extraction

## 1. Introduction

Dufulin is a new α–aminophosphonates antiviral agent developed by Guizhou University of China recently. It displays a high antiviral activity towards tobacco mosaic disease but has a low acute toxicity to livestock and human beings.[1] The conventional pretreatment methods for analyzing residual Dufulin in environments were liquid–liquid extraction, florisil catridges and solid–phase extraction.[2] However, the traditional separation and purification techniques always exhibit low–separation efficiency and consume a large quantity of organic solvents, with tedious operations, potential impurity introduction and analyte loss.[3] Therefore, there is a need to develop a highly sensitive, reliable and selective method to determine Dufulin at a trace level.

Molecular imprinting is an excellent technique due to its selective recognition sites in a stable polymer matrix. The synthesis of molecularly imprinted polymers (MIPs) involves formation of template–monomer complexes through either covalent or noncovalent interactions, followed by a copolymerization with cross–linking agent. Thus, a rigid and highly cross–linked macroporous polymer is formed. After removal of templates from the cross–linked matrix, MIPs generate the recognition cavities complementary to the shape, size, and functionality of templates. Therefore, MIPs can be used as an artificial receptor to selectively rebind target molecules from a mixture of chemical species.[4,5] Due to their high stability, easy preparation and flexible application, MIPs have become a particularly attractive material.[6-8] To date, MIPs have been widely used in many areas including chromatography stationary phase for separation purpose,[9,10] solid–phase extraction,[11,12] dispersive solid–phase extraction,[13] electrochemical sensors,[14,15] membrane separation,[16] solid–phase

microextraction[17,18] and drug delivery systems.[19]

However, molecule imprinting polymers prepared by conventional methods do have some limitations including incomplete template removal, slow mass transfer, small binding capacity, poor site accessibility and irregular materials shape, all of which result from the fact that the templates located at interior area of materials were extremely difficult to extract.[8,20] To solve the problems, Yilmaz and co-workers (2000)[21] first reported the surface molecular–imprinting strategy by covalent immobilization of template molecules at the surface of solid substrates. MIPs prepared by this method have small dimension with extremely high surface–to–volume ratio, so most of template molecules are situated for the surface of imprinted materials, providing a complete removal of the templates, an excellent accessibility to target species and a low resistance of mass transfer.[22,23]

Hydrogen bonding between template and functional monomers are easily destroyed in aqueous media because aqueous solvents compete over adsorption with the template for functional monomers.[24] The molecular structure of β–cyclodextrin allows it to create a lipophilic inner cavity with hydrophilic outer surfaces, which is capable of interacting with a large variety of guest molecules to form non-covalent inclusion complexes. In this regard, β–cyclodextrin and its derivatives have been chosen as functional monomers to achieve molecular imprinting in aqueous solution.[25,26] To the best of our knowledge, no literature has been available on the MIP coupled with solid phase extraction (SPE) to determine Dufulin in environmental samples. In this study, a newly developed method was described to synthesize the molecular imprinted polymers of Dufulin on the surface of silica gel. The Dufulin–imprinted polymers were evaluated by conducting a series of binding experiments.

The polymers were used as a material in SPE for analysis of Dufulin in different environmental matrix.

## 2. Experimental

### *2.1. Materials and chemicals*

The pesticide Dufulin was obtained from Center for Research and Development of Fine Chemicals of Guizhou University, with purity of 99%. Diazinon (Dia) was purchased from Nantong Jiangshan Agrochemical & Chemicals Co., Ltd. Malathion (Mal) and Isoproturon (Iso) were obtained from the Academy of Agricultural Science in Jiangsu, China. The purity of Dia, Mal and Iso were 96, 95 and 97%, respectively. Silica gel (40-45 μm) was purchased from Qingdao Ocean Chemical Co., Ltd. γ–Glycidoxypropyltrimethoxysilane (GOTMS) and 3-methylacryloxypropyltrimethoxysilane (MATMS) were purchased from Nanjing Xiangqian Chemical Co., Ltd. Ethyleneglycol dimethacrylate (EGDMA) was purchased from Shanghai Jiachen Chemical Co., Ltd. All other chemicals were provided by Nanjing Chemical Reagent Co., Ltd. β–Cyclodextrin (β–CD) was recrystallized and dried under vacuum at 110 °C for 24 h. Azodiisobutyronitrile (AIBN) was recrystallized by methanol. N, N–dimethylformamide (DMF) was dried over 3 Å molecular sieves.

Soil samples were collected from the surface layer at the Experimental Station of Nanjing Agricultural University, Nanjing. The soil was air–dried and sieved through a 2 mm sieve mesh prior to use. Wheat seeds were obtained from the Academy of Agricultural Science in Jiangsu, China, and cultivated under laboratory conditions.

### *2.2. Instruments and operation parameters*

A TENSOR–27 FT–IR spectrometer (Bruker, Germany) with a resolution of 2 $cm^{-1}$ and a spectral range of 4000–400 $cm^{-1}$ was employed to examine FT–IR spectra of samples by a pressed tablet (sample: KBr = 1: 100, *w:w*). The morphologies and structures of the samples were examined using a JSM–6380 LV SEM at 30 kV (JEOL, Japan). Areas of samples were magnified to 5000 folds. All chromatographic measurements were performed using a high performance liquid chromatography (HPLC) system (Waters 2489, Waters Technologies Co. Ltd.), equipped with a 515 pump and a UV–vis detector. A $C_{18}$ column (250 mm×4.6 mm i.d., 5 μm) was taken as the analytical column used at room temperature. The mobile phase was methanol/water (75/25, *v/v*) with detection at 235 nm and a flow rate of 0.6 mL $min^{-1}$. The injection volume was 20 μL.

### *2.3. Preparation of Dufulin molecularly imprinted polymer*

#### *2.3.1. Activation of silica gel*

Twenty grams of silica gel were mixed with 100 mL of hydrochloric acid/deionized water (1/9, *v/v*) and refluxed with continuous stirring for 12 h. The silica gel particles was collected, washed with double–distilled water until the pH was neutral and dried under vacuum.

#### *2.3.2. Synthesis of functionalized silica gel*

The process to prepare Dufulin–MIPs is shown in Supplementary Fig. S1. First, β–CD

(2.29 g) was dissolved in 50 mL of anhydrous DMF and 0.3 g NaH was sequentially added with continuous stirring. When no gas was emitted, excessive NaH was removed by filtration. GOTMS (1.0 mL) was added to the filtrate, and the mixture was stirred under nitrogen atmosphere at 90 °C for 5 h. After that, 8 g activated silica gel particles, 50 mL anhydrous DMF and 1.0 mL of MATMS were added to the above reaction system, which continued to be stirred under nitrogen atmosphere at 110–120 °C for 24 h. The product (β–CD–silica gel, CDS) was washed several times with anhydrous DMF, methanol, distilled water and acetone respectively and finally dried under vacuum at 80–90 °C.[27]

After 2.0 g of 4–toluene sulfonyl chloride was dissolved completely in 50 mL anhydrous pyridine, CDS (2.0 g) was added to the solution. The mixture was stirred at room temperature for 24 h, followed by 2–3 °C for 18 h. The product (4–toluene sulfonyl–CDS, TsyCDS) obtained and was successively washed with anhydrous pyridine, ether, methanol and distilled water and dried under vacuum at 100 °C.[24]

The functionalized silica gel particles (F–silica gel, DCDS) were synthesized by the following steps: 2.0 g 2, 4–Dichlorophenol was dissolved in 50 mL of anhydrous DMF, to which 2.0 g NaH was added. The mixture was stirred at room temperature until no gas was emitted and filtered. TsyCDS (3.0 g) was added to the filtrate and the mixture was stirred at 80–90 °C under nitrogen protection for 24 h. DCDS was filtered and washed several times, successively with anhydrous DMF, anhydrous ethanol, methanol, distilled water and acetone. Finally, DCDS was dried under vacuum at 70 °C. The F–silica gel of HCDS was prepared using the same way except that 2, 4–dichlorophenol was replaced by 7–hydroxycoumarin.

155 ***2.3.3. MIPs preparation***

156 A series of molecularly imprinted and non–imprinted polymers were prepared using
157 anhydrous DMF as porogen according to the amounts presented in Supplementary Table S1.
158 DCDS was used as supporting matrix for MIP–2, MIP–3 and MIP–4, CDS for MIP–1, and
159 HCDS for MIP–5.

160 The Synthesis procedure of MIP–2 was described as follows: prior to polymerization, the
161 prearranged solution was prepared by dissolving Dufulin (1 mM), functional monomer
162 methacrylic acid (MAA, 4 mM) and DCDS (2 g) into 30 mL anhydrous DMF in a glass tube,
163 and stirring at room temperature for 4 h. The cross-linking agent EGDMA (15 mM) and
164 initiator AIBN (60 mg) were added to the above solution. The mixed solution was purged with
165 nitrogen gas for 10 min. The polymerization was carried out in a water bath at 50 ℃ for 6 h
166 with constant stirring, and then this reaction was conducted at 60 ℃ for 24 h to obtain a high
167 cross–linking density. MIP–2 was separated and cleaned by a mixture of methanol and acetic
168 acid (9:1, *v/v*) in a Soxhlet extraction apparatus until no Dufulin was detected in the washing
169 solution by HPLC. Finally, MIP–2 was washed with excess of deionized water until pH of the
170 washing solution reached to 7.0 and dried under vacuum. NIP–2 was prepared in the same
171 way with no addition of Dufulin. MIP–3 (NIP–3) was prepared using acrylamide (AM) as a
172 functional monomer, and MIP–4 (NIP–4) using the mixture of MAA and AM (mole ratio 1:1).

173

174 ***2.4. Adsorption experiments***

175 ***2.4.1. Measurement of adsorption isotherm***

176 Briefly, 10 mg of polymer particles MIP–2 was mixed with 5 mL of methanol:water (3/7,

177 *v/v*) solution at Dufulin concentration ($C_0$) of 0.01–0.15 mM in each centrifuge tube. The
178 centrifuge tubes were shaken at 25 ℃ for 24 h. The mixture was centrifuged for 10 min
179 (10,000 *g*) and filtrated through a 0.45 μm filter. Concentration ($C_e$) of Dufulin in supernatant
180 was determined by HPLC. The adsorption isotherm of NIP–2 was detected following the
181 above method. The equilibrium amount of substrate bound to the polymer ($Q_e$) was calculated
182 according to Eq.1:

183 $$Q_e = \frac{MV(C_0 - C_e)}{m} \quad (1)$$

184 Where $Q_e$ (mg $g^{-1}$) is the adsorption amount; V (mL) is the volume of the Dufulin
185 solution; m (mg) is the weight of the polymer particles; and M (g $mol^{-1}$) is the molar mass of
186 the template.

187

188 ***2.4.2. Measurement of kinetic adsorption curve***

189 Kinetic adsorption was carried out using 10 mg MIP–2 at the Dufulin concentration of
190 0.12 mmol $L^{-1}$ in 5 mL of methanol:water (3/7, *v/v*) solution. The mixture was shaken at 25 °C.
191 At different time intervals, the concentration of Dufulin in the supernatants was determined
192 by HPLC. The kinetic adsorption of NIP–2 was carried out the same as MIP–2.

193

194 ***2.4.3. Selectivity experiment***

195 The molecular selectivity was further investigated by testing the binding capacities of
196 MIP–2 towards Dufulin (Duf), Diazinon (Dia), Malathion (Mal) and Isoproturon (Iso). The
197 mixed solution (methanol:water (3/7, *v/v*)) of four pesticides was prepared, in which the
198 concentration of Dufulin was 0.12 mmol $L^{-1}$ (same for other three components). Ten mg

MIP–2 and 5 mL of mixed standard solution were placed in a 10 mL centrifuge tube. These mixtures were shaken at 25 °C for 24 h and centrifuged for 10 min (10,000 *g*). The supernatants were diluted and concentrations of Duf, Dia, Mal and Iso in diluted solutions were determined by HPLC.[28] NIP–2 was the control to compare with the selectivity of MIP–2. Distribution coefficients $K_d$ (mL $g^{-1}$) of four pesticides were calculated by Eq.2:

$$K_d = \frac{Q_e}{C_s} \quad (2)$$

Where $Q_e$ (mg $g^{-1}$) represents the equilibrium amount adsorbed; $C_s$ is the equilibrium concentration. The selectivity coefficient k of MIP–2 can be obtained from the equilibrium binding data according to Eq.3:

$$k = \frac{K_d(\text{template})}{K_d(\text{analogue})} \quad (3)$$

Furthermore, the value of the relative selectivity coefficient k' was calculated according to Eq.4:[29]

$$k' = \frac{k_{MIP}}{k_{NIP}} \quad (4)$$

### *2.5. Method validation and application to real samples*

The calibration curve was constructed by measuring the mixed standard solutions of Dufulin, Diazinon, Malathion and Isoproturon in five different concentrations ranging from 1 to 20 mg $L^{-1}$. The detection limit of instrument (D) was defined as three times ratio of signal to noise. The method limit of detection (LOD) was also defined as three times ratio of signal to noise and was conducted by measuring the elution of Dufulin solution after MIP–SPE in real samples.

For assessment of accuracy and precision, the real samples spiked with Dufulin were tested. Tap water samples with Dufulin at 0.01, 0.1 and 0.5 mg $L^{-1}$ were prepared by spiking standard Dufulin, respectively. Soil samples (10 g) and homogenates of wheat (5 g) were mixed with 1 mL of methanol solution, in which the concentrations of Dufulin were 0.1, 0.5 and 1 mg $kg^{-1}$, respectively.

MIP–2 particles (200 mg) were packed in an empty solid phase extraction (SPE) cartridge and used for MIP–SPE, in which the PTFE frits were placed on both top and bottom. The MIP–SPE cartridge was washed with 10 mL methanol: acetic acid (9/1, *v/v*) to remove residues and conditioned with 5 mL methanol and deionized water, respectively. The MIP–SPE column was attached to a vacuum manifold apparatus for analysis of environment samples.

Water sample (20 mL) was loaded onto the conditioned MIP–SPE cartridge at a speed of 0.5 mL $min^{-1}$ and residual water on the column was removed by maintaining a negative pressure for 5 min. Subsequently, the MIP–SPE cartridge was washed with 4 mL methanol:water (75/25, *v/v*). Eluate was collected and filtered through a 0.45 μm filter before HPLC analysis. Soil samples (10 g) and homogenates of wheat (5 g) were sonicated and extracted with 20 mL of acetone:water (3/1, *v/v*) by a ultrasonic cleaner for 30 min. The extraction procedure was performed in triplicate. The supernatant was concentrated by a rotary evaporator to remove acetone at 40 °C. The residue water was loaded onto the conditioned MIP–SPE column at a speed of 0.5 mL $min^{-1}$. The eluate was discarded. The column was washed with 4 mL methanol:water (75/25, *v/v*). The eluate was collected, filtered through a 0.45 μm filter and analyzed by HPLC. Each sample was repeated in triplicate.

### *2.6. Classical sample preparation technique*

The classical sample preparation for extraction of Dufulin in the environmental samples were established and performed as follows: Tap water (20 mL) was loaded onto the conditioned commercial C18 solid phase extraction column (SUPELCO, 3 mL, 500 mg) at a speed of 0.5 mL $min^{-1}$ and the elutate was discarded. Then 4 mL methanol:water (75/25, *v/v*) was used to elute the C18 solid phase extraction column (SPE). The eluate was collected and filtered through a 0.45 μm filter before HPLC analysis. The extraction of soil sample (10 g) and homogenates of wheat (5 g) were the same as section 2.5. The clean-up procedure was performed by liquid-liquid extraction and column chromatography: the residue water after rotary evaporator was transferred into a separatory funnel and extracted by petroleum ether for three times, each time with 15 mL. The organic phase was collected and evaporated to dryness by a rotary evaporator at 40 ºC. The residue was redissolved in 3 mL petroleum ether and then transferred to a glass column containing 5 g of activated silica gel. The column was washed with 15 mL acetone:petroleum ether (3:7, *v/v*), and elutes were discarded. The glass column was re-washed with 30 mL acetone. The washing solution was collected and completely dried by a rotary vacuum evaporator at 40 ºC. The residue was re-dissolved in 4 mL methanol:water (75/25, *v/v*) for HPLC analysis.

## 3. Results and discussion

### *3.1. Characterization of FT–IR and SEM*

To confirm the modification on the surface of silica gel and preparation of MIP, FT–IR spectra of activated silica–gel (a), CDS (b), DCDs (c) and MIP–2 (d) were obtained. The peaks at 3369.26 $cm^{-1}$ and 1634.71 $cm^{-1}$ correspond to the vibration of hydroxyl group (Fig. 1A-a). The observations around 1087.27, 798.68 and 472.40 $cm^{-1}$ indicated the Si–O–Si and Si–O–H stretching vibrations, respectively.[27] Compared with FT–IR spectra of activated silica–gel (Fig. 1A-a), CDS and DCDS displayed unique peaks of carbonyl group of MATMS at 1722.35 $cm^{-1}$; the peaks around 2991.41 and 2940.83 $cm^{-1}$ represent the typical feature of β–CD (Fig. 1A-b and 1A-c). These new bands indicated that GOTMS bonded β–CD and MATMS were modified on the surface of silica gel. The feature at 1729.92 $cm^{-1}$ was attributed to the carbonyl groups of the functional monomer MAA (Fig. 1A-d), indicating that MIP–2 was successfully prepared.

SEM was used for characterizing activated silica–gel (a), DCDS (b), NIP–2 (c) and MIP–2 (d) (Fig. 1B). The activated silica–gel displayed a smooth surface (Fig. 1B-a). For DCDS, no significant change was observed compared with the silica gel (Fig. 1B-b), which was likely due to the fact that the modified molecule was too small. The morphology of NIP (Fig. 1B-c) and MIP (Fig. 1B-d) was different from silica–gel (Fig. 1B-a) and DCDS (Fig. 1B-b), and the three-dimensional structure of NIP and MIP was possibly caused by polymerization. Because the polymer generated cannot dissolve in the polymerization mixture, a porous and loose structure was formed. Due to the absence of template, the polymer formed in NIP was merely by the radical polymerization of functional monomer, and the image of NIP showed a rough surface with some irregular pores. The image of MIP appeared rougher and looser because of fixation of the prearranged polymer of template and functional

monomer on the surface of silica-gel in the presence of crosslinker, porogen and initiator in the polymerization system.

### *3.2. Adsorption performance of MIPs*

#### *3.2.1. Binding capacity of MIPs*

MIPs synthesized by non–covalent imprinting approach were rapidly recognized, so the simplicity of the synthesis process can be obtained.[30] Considering the chemical structure of template Dufulin, the functional monomer that has carbonyl group was suited to interact each other, and MAA and AM were chosen as functional monomers (Supplementary Table S1). All polymeric matrices were prepared using the same molar ratio among template, functional monomer and crosslinker.

Imprinting effects in the synthesized materials were evaluated by binding experiments in which a certain amount of polymeric particles was incubated with the Dufulin solution of 0.12 mmol $L^{-1}$ for 24 h. As shown in Supplementary Table S1, all Dufulin–imprinted polymers have much higher adsorption capacity for the template than the corresponding referenced polymers (NIPs). Among the five MIPs, MIP–2 showed the highest binding capacity for template Dufulin, indicating that MIP–2 offered a higher affinity for the template molecule. MIP–2 was synthesized using F–silica gel (DCDS) as supporting matrix, which was the derivative of 2, 4–dichlorophenol, GOTMS and MATMS with β–cyclodextrin bonded silica gel (Supplementary Fig. S1).

Qu et al. (2012) [24] reported that a MIP with a specific capability for detecting the template was obtained, when 2, 4, 6–trinitrophenol (PA) was used to modify β–cyclodextrin

bonded to the silica gel. In MIP–2 preparation, 2,4,6–trinitrophenol (PA) was substituted by 2,4–dichlorophenol, a π–acidic substance. When 2,4–dichlorophenol was applyed to modifying β–cyclodextrin, not only the effect of π–π sites was increased, but also the mouth space of β–cyclodextrin was changed. As a consequence, the separation and selectivity of β–cyclodextrin were altered for Duflin. 7–Hydroxycoumarin was used to modify β–cyclodextrin in MIP–5. But β–cyclodextrin was not modified in MIP-1, indicating that 2,4–dichlorophenol played an important role in the binding effect for Dufulin, in comparison with MIP–2 with MIP–1 and MIP–5 (Supplementary Table S1).

In order to investigate the role of functional monomer, different MIPs (MIP–2, MIP–3 and MIP–4) were prepared using MAA, AM or their mixture respectively. MIP-2 had the highest adsorption capacity (17.45 mg $g^{-1}$), suggesting that MAA was an effective functional monomer when Dufulin was used as a template (Supplementary Table S1). The result may be that hydrogen bond was formed between the hydroxyl of functional monomer MAA and fluorine or phospholipid groups in Dufulin. Additionally, the hydrophobic effect of β–cyclodextrin could be the reason for recognizing Dufulin. Therefore, MIP–2 was selected for the further investigation.

### *3.2.2. Effect of adsorption medium*

The adsorption medium affected microenvironment of adsorption and stability of analyte simultaneously.[31] In order to evaluate the effect of solvent on adsorption amount, solutions with different ratios of methanol and $H_2O$ (30:70, 35:65, 40:60, 45:55, *v/v*) were prepared with Dufulin at 0.12 mmol $L^{-1}$. With less than 30% (*v/v*) of methanol in the mixture, the

polymers were not properly tested. Compared with NIP–2, MIP–2 always showed higher adsorption for Dufulin. When the ratios of methanol and $H_2O$ were 30:70, 35:65, 40:60 and 45:55 (*v/v*), the adsorption amount of MIP–2 for Dufulin was 17.45, 15.79, 13.58 and 7.20 mg $g^{-1}$ respectively. The adsorption amount of MIP–2 was increased with the proportion of $H_2O$ from 55 to 70% (*v/v*), whereas the change of methanol proportion in solution had much less influence on the binding performance of NIP–2. Moreover, the cavity of β–CD was relatively hydrophobic compared to water and template, so that the hydrophobic effect increased as the $H_2O$ content increased in the solution and more template molecules could be driven into the cavities of the polymers. Our analysis was consistent with the previous report.[25,31] The binding of Dufulin to MIP–2 was caused not only by the insertion of Dufulin into cavities formed during the imprinting process, but also by unspecific interactions between Dufulin and the polymer, while the binding of Dufulin to NIP–2 was caused only by unspecific interactions.

### *3.2.3. Adsorption isotherm*

The binding capacity of Dufulin on MIP–2 was an important parameter to estimate how much MIP was required to bind a specific amount of Dufulin from solution. For this purpose, the binding isotherms were determined in the initial concentrations of Dufulin ranging from 0.01 to 0.15 mmol $L^{-1}$. The adsorption amount of MIP–2 toward Dufulin increased progressively with the increment of Dufulin, resulting from the tailor–made recognition cavities during the imprinting process (Fig. 2a). When the concentration of Dufulin was up to 0.12 mmol $L^{-1}$, the adsorption was saturated. The adsorption capacity of MIP–2 (17.59 mg $g^{-1}$)

352 was about 5.1 fold over that of NIP–2 (3.50 mg $g^{-1}$) with 0.15 mmol $L^{-1}$ Dufulin.

353 Analysis of binding isotherm can be performed by Langmuir isothermal (5), Freundlich
354 isothermal (6) and Langmuir-Freundlich isotherm equation (7), respectively:

355 $$\frac{C_e}{q_e} = \frac{C_e}{Q_m} + \frac{1}{Q_m K_L} \quad (5)$$

356 $$\log q_e = \left(\frac{1}{n}\right)\log C_e + \log K_F \quad (6)$$

357 $$\mathrm{B} = \frac{N_t a F^m}{1 + a F^m} \quad (7)$$

358 Where $q_e$ (mg $g^{-1}$) and $C_e$ (mg $L^{-1}$) are the amount adsorbed on MIP–2 and concentration
359 of Dufulin in the solution at equilibrium; $K_F$ (L $mg^{-1}$) and $n$ is determined from a linear plot of
360 log $q_e$ versus log $C_e$, which are Freundlich constants demonstrating adsorption capacity and
361 intensity, respectively; $Q_m$ (mg $g^{-1}$) is the theoretical maximum adsorption capacity; $K_L$ (L
362 $mg^{-1}$) is the Langmuir constant related to the affinity of adsorption sites; $N_t$ is the total number
363 of binding sites; $a$ is related to the median binding affinity constant $K_0$ ($K_0 = a^{1/m}$); and $m$ is
364 the heterogeneity index. For a homogeneous material, $m$ is equal to 1, whereas when $m$ is
365 within 0 and 1, the material is heterogeneous.

366 The corresponding parameters fitting Freundlich, Langmuir and Langmuir-Freundlich
367 isotherm models to the experimental data were obtained (Fig. 2b, 2c and 2d). The values of
368 $Q_m$ and $K_L$ were 26.31 mg $g^{-1}$ and 0.041 L $mg^{-1}$, respectively. The values of $n$ and $K_F$ were
369 0.9382 and 1.0012 L $mg^{-1}$, respectively. The values of $N_t$, $K_0$, $m$ and $a$ were 90.83 μmol $g^{-1}$
370 (37.09 mg $g^{-1}$), 3.974 $M^{-1}$, 0.979 and 3.339 $M^{-1}$, respectively. The Langmuir isothermal
371 equation with $R^2$ value of 0.9216 seemed more desirable to describe Dufulin adsorption on
372 MIP–2 than the Freundlich isothermal equation with $R^2$ value of 0.8862 and

Langmuir-Freundlich equation of 0.902. Furthermore, the Langmuir-Freundlich presents a more general case that encompasses both Langmuir and Freundlich models. When $m$ =1, the Langmuir-Freundlich isotherm is homogeneous to the Langmuir isotherm. The value of m (0.979 to 1) confirmed that the Langmuir model was applicable to MIP-2. All results above indicated that Dufulin bound to MIP–2 was most likely a unimolecular adsorption with the typical characteristic of chemical adsorption, which was in agreement with the previous report.[32,33]

### *3.2.4. Adsorption kinetic*

Binding kinetic of the template Dufulin with MIP–2 and NIP–2 was also evaluated. Before adsorption equilibrium was reached, MIP–2 could bind Dufulin molecules from solution phase at a much faster rate than the polymer NIP–2 (Fig. 3). MIP–2 took up 90% of equilibrium absorption amount for only 60 min, with 240 min reaching to adsorption equilibrium, whereas the NIP–2 adsorption amount for Dufulin did not change obviously with the time. There was a four–fold increase in adsorption capacity with MIP–2 over NIP–2 (Fig. 3). Thus, the adsorption process could be divided into two phases: the rapid adsorption in the first 60 min and slow adsorption thereafter. The binding sites of MIP–2 were suggested at the surface or in the proximity of the surface. Most of the template could be access to the imprinted site at a high rate of speed. Taken together, MIP–2 showed the high binding capacity and fast kinetic adsorption, and was favorable to be used for the pretreatment of environmental samples by solid phase extraction (SPE).

395 ***3.2.5. Adsorption selectivity***

396 The molecular selectivity of MIP–2 was investigated using Mal and Dia as structural
397 analogues of Dufulin template and Iso as reference compound in the mixture solution with
398 0.12 mmol $L^{-1}$ pesticides. The polymer NIP–2 was used as comparison. The MIP–2 binding
399 capacity to Dufulin was about 2.18, 2.50 and 7.36 times that of Mal, Dia and Iso, respectively
400 (Fig. 4a), indicating that the polymer MIP–2 possessed high selectivity to Dufulin template
401 than its structural analogues. This high selectivity was mainly attributed to the molecular size
402 recognition of MIP–2 to template molecule and the hydrogen bonding interactions between
403 the hydroxyl of functional monomer MAA and phospholipid groups in Duf at specific
404 positions. Because the structures of Mal and Dia were similar to the template molecule Duflin,
405 the binding capacities of Mal and Dia (16.10 and 14.05 μmmol $g^{-1}$) for the MIP–2 were higher
406 than that of Iso (Fig. 4). From the chemical structure, the Mal molecule possesses a smaller
407 spatial diameter than Dia, which could make Mal much easier to enter the imprinted cavities
408 of Dufulin. Therefore, MIP–2 exhibited a larger binding affinity to Mal than Dia. In contrast,
409 Iso had a little structural similarity with Duf. So the binding capacity of Iso was lowest among
410 the four pesticides. The main reason for it could be the mismatch of its structure and size with
411 the specific cavities on the surface of MIP–2. However, NIP–2 did not show the obvious
412 difference in the binding capacities of Duf, Mal, Dia, and Iso. The binding effect of NIP-2 for
413 the four pesticides was likely dependent on the same mechanism of nonspecific absorption.

414 As for the selectivity experiments to MIPs, we also chose different ratios of interferences
415 (Iso, Mal and Dia) and Dufulin to determine whether the concentrations of the interferences
416 affect the ability to extract Dufulin. The different ratios of interferences and Dufulin were set

as following: interferences/dufulin (mmol $L^{-1}$/mmol $L^{-1}$) were 0.5/1, 1/1, 2/1 and 3/1. When the concentration of interferences was 1/2 of Dufulin, the polymer had a higher extraction of Dufulin with an adsorption amount of 41.93 μmol $g^{-1}$ (Fig. 4b). The higher concentrations of interferences did not substantially affect the ability to extract Dufulin. When the concentrations of interferences reached to three folds of Dufulin, the concentration of Dufulin adsorbed was 31.01 μmol $g^{-1}$, that was 88.3% of the value adsorbed as the interferences had an equal concentration to Dufulin (Fig. 4b).

The distribution coefficient ($K_d$), selectivity coefficient of the sorbent ($k$) and relative selectivity coefficient ($k'$) were calculated by equation (2)–(4). $K_d$ represents the ratio of the binding amount of sorbent to free analyte concentration in the supernatant. $k$ of sorbent indicates the difference between the two substances adsorbed by one sorbent. $k'$ represents the difference between the two sorbents.[34,35] $k$ of NIP–2 for Iso, Mal and Dia were very low, which were 1.79, 0.76 and 1.03, respectively. $k$ of MIP–2 showed a more significant increase than the values of NIP–2, due to the imprinting effect. The value of $k'$ is an indicator of adsorption affinity for recognition sites to the template Dufulin. It is shown that $k'$ values ranged from 4.47 to 9.11 (greater than 1) (Table 1) and MIP–2 had higher selectivity than NIP–2. These results indicated that Dufulin can be bound to MIP–2 even in the presence of Isoproturon, Malathion and Diazinon interferences.

### *3.3. Method validation and application to real samples*

Determination of Dufulin, Isoproturon, Malathion and Diazinon with HPLC was carried out as described above. The linearity of the calibration curves were obtained by identifying

the peak areas from analysis of 1 to 20 mg $L^{-1}$ of each analyte (Supplementary Table S2). The good linearity was achieved and all $R^2$ –values were higher than 0.997. The detection limits of instrument (D) for the four analytes ranged from 0.002 to 0.075 mg $L^{-1}$ (Supplementary Table S2).

The accuracy of the method was estimated by determining tap water, soil and wheat samples spiked with Dufulin at three different concentration levels. As shown in Fig. 5A, the MIP–SPE column was able to pre-concentrate analyte, and the average recovery of the spiked concentrations of Dufulin in water was 88.98–102.16%, with the RSD less than 0.75–2.59%. The limit of detection (LOD) in water was 0.0008 mg $L^{-1}$ (Table 2). With regard to soil sample, the MIP-SPE columns were used to remove the interference and re–concentrate Dufulin. Although there were some impurities after soil sample was treated by MIP-SPE, they had the least impact on the Dufulin detection (Fig. 5B). The complexity of the blank wheat sample background was evident and the interfering substances would influence the quantitative determination of Dufulin (Fig.5C-a). Compared with the direct HPLC analysis without pretreatment of MIP-SPE, interference in the blank wheat sample was successfully cleaned up after MIP-SPE (Fig.5C-b). This allowed the extraction of Dufulin in wheat samples to be highly selective and the quantitative analysis of Dufulin be easily coupled with HPLC (Fig.5C-c). The average recoveries of Dufulin in the spiked soil and wheat samples were 85.31–99.57% and 87.84–100.19%, and the RSDs were 1.50–4.85% and 3.87–6.25%, respectively (Table 2). The values of LOD in soil and wheat samples were 0.010 and 0.023 mg $kg^{-1}$, respectively.

Concerning applicability of the developed method, we chose tap water to estimate the

recovery of Dufulin by adding other three analytes (Iso, Mal and Dia). Water sample (20 mL) containing the mixture (Iso, Mal, Dia and Duf) at 0.10 mg $L^{-1}$ of every analyte was loaded onto the conditioned MIP–SPE cartridge. The MIP–SPE cartridge was washed with 2 mL methanol:water (55/45, *v/v*). The eluate (water) was discarded. Finally, 4 mL methanol:water (75/25, *v/v* ) was used for elution. The eluate was collected and filtered through a 0.45 μm filter before HPLC analysis. The measurements were repeated three times. Recoveries of Iso, Mal, Dia were only 16.47%, 30.56% and 28.64%, respectively, indicating that the proposed method was not applicable for Iso, Mal and Dia. However, the average recoveries of Dufulin were 89.21%.

A comparison of the results from the proposed MIP–SPE–HPLC and the classical methods for determination of Dufulin residue in water, soil and wheat tissue was shown in Table 2. The proposed method has comparable LODs and RSDs with the classical method. For soil and wheat samples, the proposed method requires shorter extraction time due to the selective binding sites at the surface of MIP. Furthermore, MIP-SPE process consumes much less toxic organic solvent and has a good clean-up and concentration effect for Dufulin. For water analysis, the developed method and existing SPE technique (with C18 SPE) seem to be very similar in time and solvent consumption. But LODs was lower than C18 SPE technique. All these results indicated that the MIP–SPE couple with HPLC was accurate and practical for selective extraction and sensitive determination of trace Dufulin in environmental samples.

## 4. Conclusions

A novel procedure has been developed to synthesize Dufulin–imprinted silica gel sorbent with a surface molecular imprinting technique. Synthetic conditions for MIP were improved. The imprinted feature of MIP was characterized by FI-IR and SEM. The binding experiments showed that MIP–2 had high affinity, capacity, and fast kinetics of adsorption to Dufulin. The equilibrium data could be fitted by Langmuir adsorption model. The MIP–2 also displayed high selectivity for Dufulin. With MIP–2 being applied as sorbent in SPE, a purification of Dufulin from environmental samples was obtained. The method of MIP–SPE coupled with HPLC showed good recoveries, high selectivity, accuracy of quantitative analysis, shorter extraction time and green safety compared with the classical method and direct determination of HPLC without MIP-SPE. The precision and accuracy of the method were satisfactory. Thus, our study represents a newly developed method for analyzing Dufulin at trace abundance in complex environmental media.

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**Appendix A. Supplementary material**

Supplementary data associated with this article can be found, in the online version

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506 **References**

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**Captions**

567 Fig. 1. FT-IR spectra (A) of activated silica-gel (a), CDS (b), DCDS (c) and MIP-2 (d). SEM
568 images (B) of activated silica gel (a), DCDS (b), NIP-2 (c) and MIP-2 (d).

569

570 Fig. 2. The adsorption isotherms (a) of Dufulin on MIP-2 and NIP-2, Freundlich plots (b),
571 Langmuir plots (c) and Langmuir-Freundlich (d) to estimate the binding nature of MIP-2.

572 Experimental conditions: 0.01–0.15 mmol $L^{-1}$ Dufulin with 10.0 mg of the polymers for 24 h,
573 and binding medium was 5 mL of methanol/water (30/70, *v/v*). The measurements were
574 repeated three times.
575
576 Fig. 3. Adsorption kinetic curves of MIP-2 and NIP-2. Experimental conditions: 0.12 mmol
577 $L^{-1}$ Dufulin in binding medium of 5 mL methanol/water (30/70, v/v) with 10.0 mg MIP-2 or
578 NIP-2 for certain hours, respectively. The measurements were repeated three times.
579
580 Fig. 4. The selective recognition property of four pesticides with MIP-2 and NIP-2 (a) and
581 MIP-2 at different ratios of dufulin and the interferences (Iso, Mal and Dia) (b). Suspending
582 10.0 mg of the polymers was in 5 mL methanol/water (30/70, v/v) for 24 h. (a) The
583 concentrations of Iso, Mal, Dia and Duf were 0.12 mmol/L. (b) The different ratios of the
584 interferences and dufulin (mmol $L^{-1}$/mmol $L^{-1}$) were 0.5/1, 1/1, 2/1 and 3/1. The
585 measurements were repeated three times. Iso:Isoproturon; Mal:Malathion; Dia:Diazinon; Duf:
586 Dufulin.
587
588 Fig. 5. Chromatograms of spiked Dufulin (0.5 mg $L^{-1}$) in water (A), soil (B) and wheat (C).
589 Samples: blank sample by direct injection (a); blank sample with MIP-SPE (b); spiked sample
590 with MIP-SPE (c).
591
592
593

Table 1

Selective recognition of MIP-2 and NIP-2 for Dufulin. Experimental conditions: suspending 10.0 mg of the polymers in 5 mL methanol/water (30/70, v/v) of mixed solution at 0.12 mmole $L^{-1}$ of every pesticide for 24 h.

| Pesticide | MIP | | NIP | | k' |
|---|---|---|---|---|---|
| | $K_d$/(mL $g^{-1}$) | k | $K_d$/(mL $g^{-1}$) | k | |
| Dufulin | 706.07 | — | 70.07 | — | — |
| Isoproturon | 43.23 | 16.33 | 39.10 | 1.79 | 9.11 |
| Malathion | 183.39 | 3.85 | 91.97 | 0.76 | 5.05 |
| Diazinon | 152.86 | 4.62 | 67.81 | 1.03 | 4.47 |

611

612 Table 2

613 The spiked recoveries of Dufulin from water, soil and wheat（*n*=3)

614

| Sample | Determined level (mg/kg) | Spiked level (mg/kg) | Proposed method | | | | Classical method | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Found (mg/kg) | Recovery (%) | RSD (%) | LOD (mg/kg) | Found (mg/kg) | Recovery (%) | RSD (%) | LOD （mg/kg） |
| Water | nd[a] | 0.01 | 0.0102 | 102.16 | 2.59 | | 0.0106 | 106.13 | 2.39 | |
| | nd[a] | 0.1 | 0.1008 | 100.79 | 0.75 | 0.0008 | 0.0961 | 96.13 | 6.98 | 0.007 |
| | nd[a] | 0.5 | 0.4449 | 88.98 | 1.58 | | 0.4737 | 94.73 | 1.26 | |
| Soil | nd[a] | 0.1 | 0.0853 | 85.31 | 1.5 | | 0.0997 | 99.67 | 4.52 | |
| | nd[a] | 0.5 | 0.4938 | 98.76 | 1.56 | 0.010 | 0.4739 | 94.78 | 3.6 | 0.013 |
| | nd[a] | 1 | 0.9957 | 99.57 | 4.85 | | 0.9147 | 91.47 | 1.05 | |
| Wheat | nd[a] | 0.1 | 0.0955 | 95.50 | 6.25 | | 0.1057 | 105.71 | 2.63 | |
| | nd[a] | 0.5 | 0.5009 | 100.19 | 3.99 | 0.023 | 0.4577 | 91.55 | 1.14 | 0.013 |
| | nd[a] | 1 | 0.8784 | 87.84 | 3.87 | | 0.9343 | 93.43 | 6.37 | |

615 [a] Not detect

616

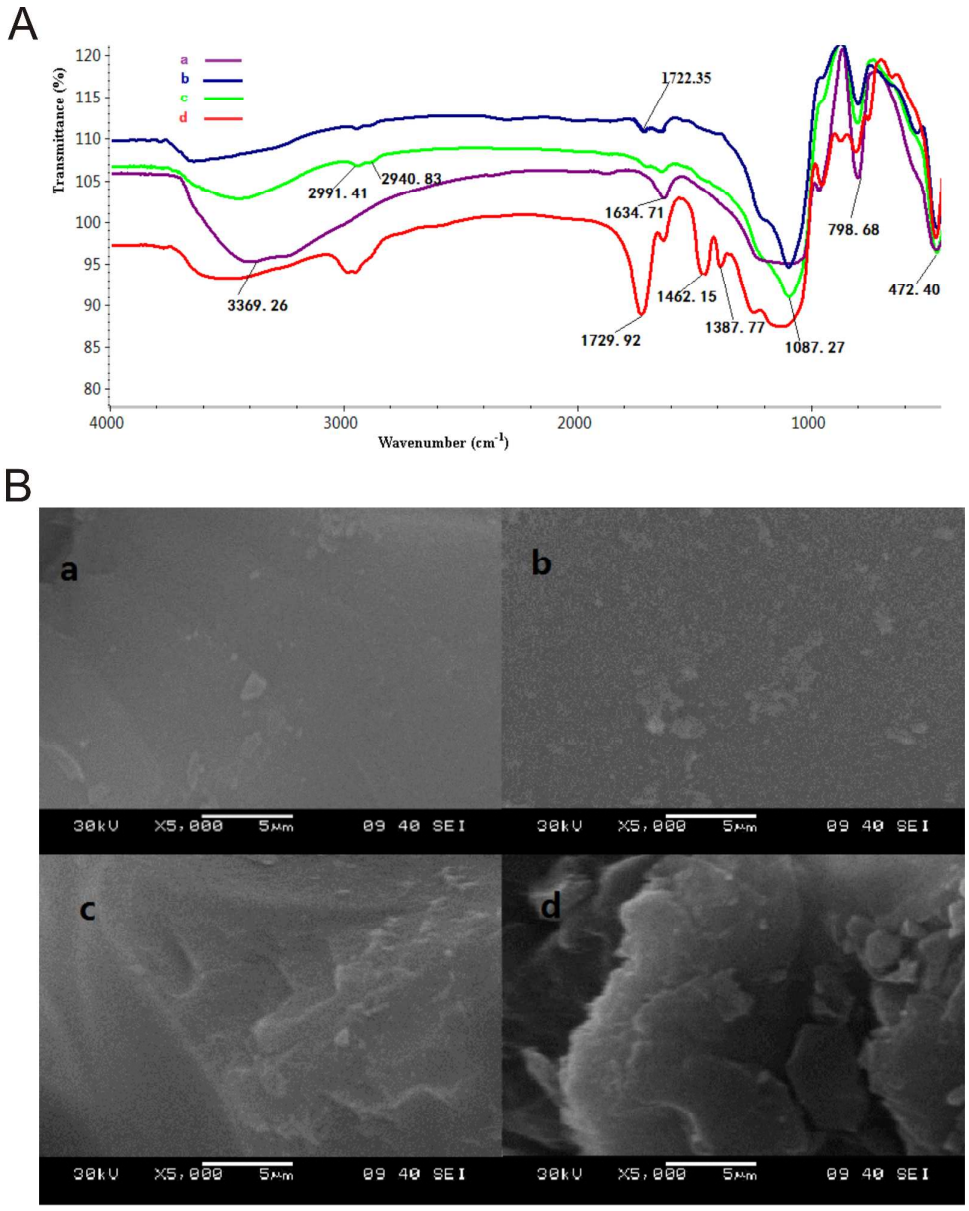
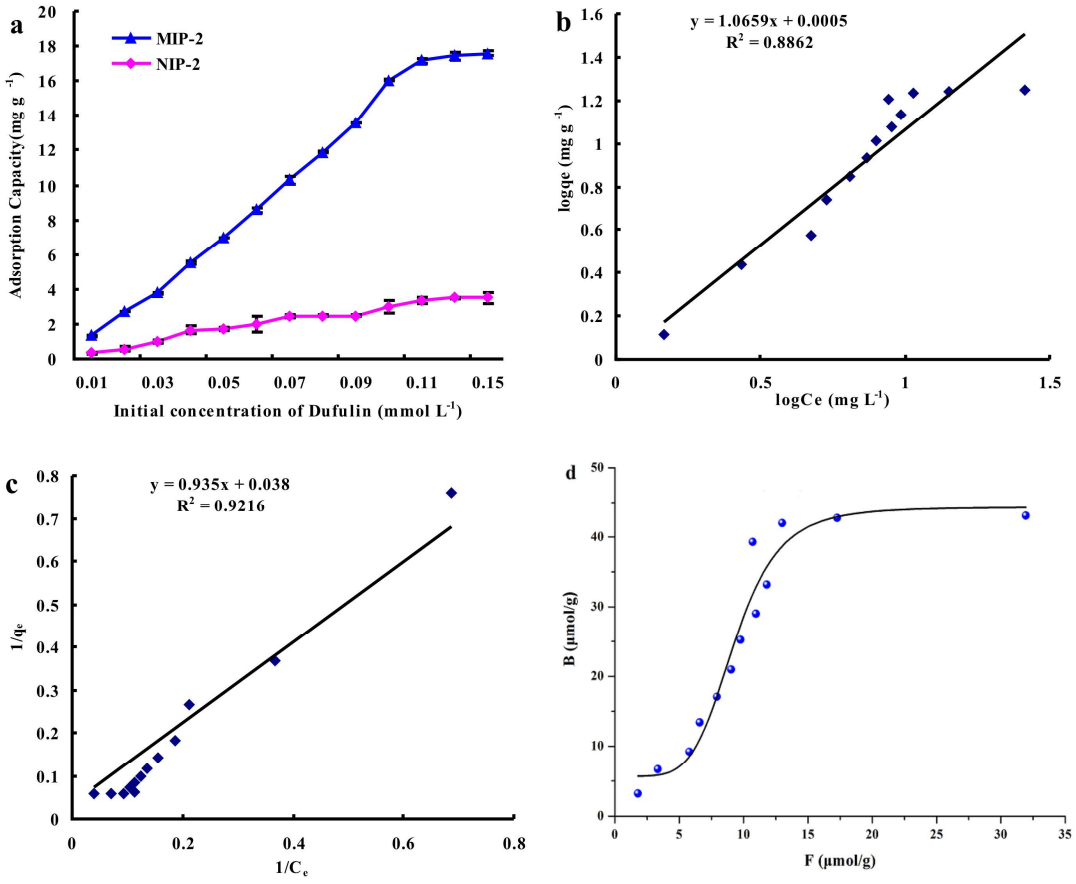



Fig. 1. FT-IR spectra (A) of activated silica-gel (a), CDS (b), DCDS (c) and MIP-2 (d). SEM images (B) of activated silica gel (a), DCDS (b), NIP-2 (c) and MIP-2 (d).

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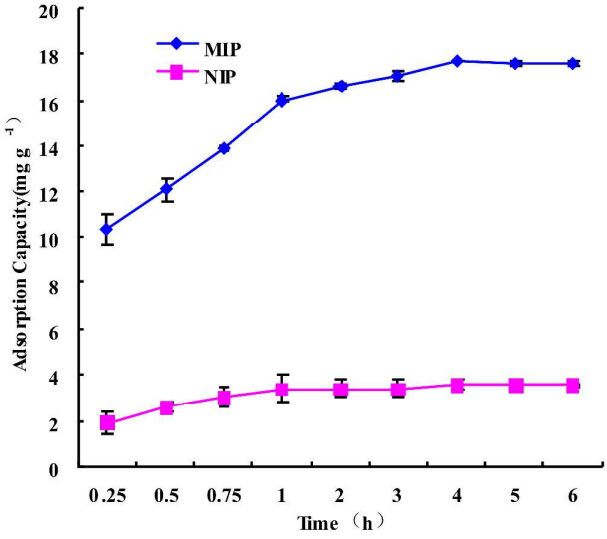
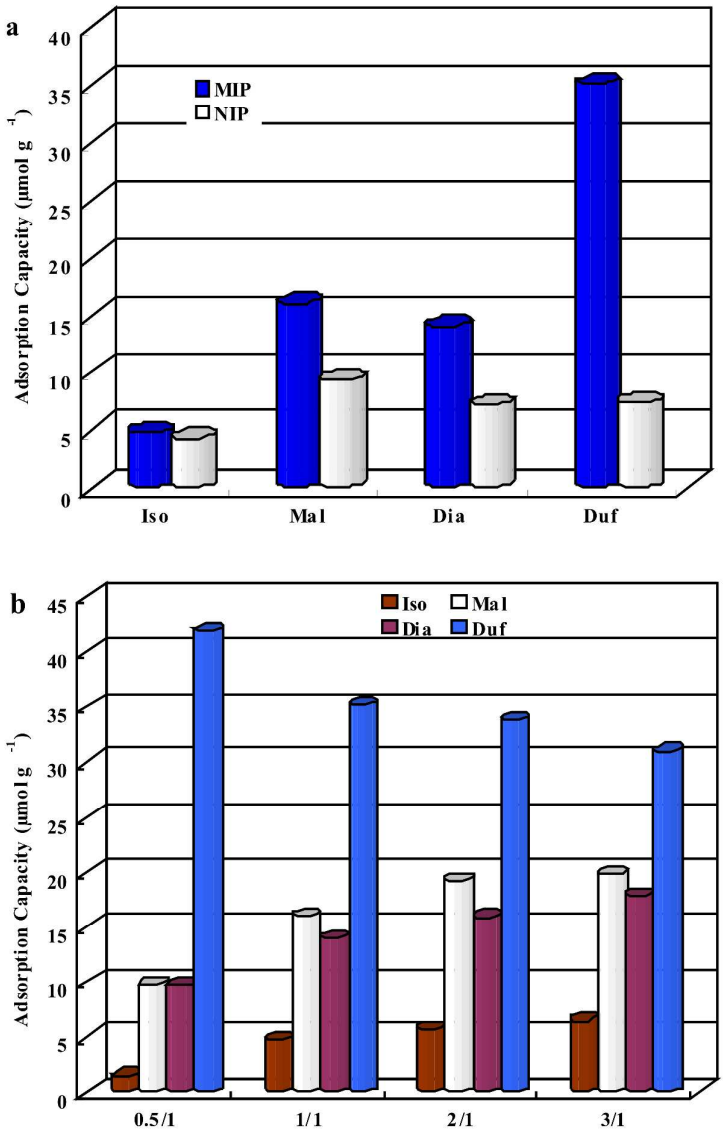



Fig. 3. Adsorption kinetic curves of MIP-2 and NIP-2. Experimental conditions: 0.12 mmol $L^{-1}$ Dufulin in binding medium of 5 mL methanol/water (30/70, v/v) with 10.0 mg MIP-2 or NIP-2 for certain hours, respectively. The measurements were repeated three times.

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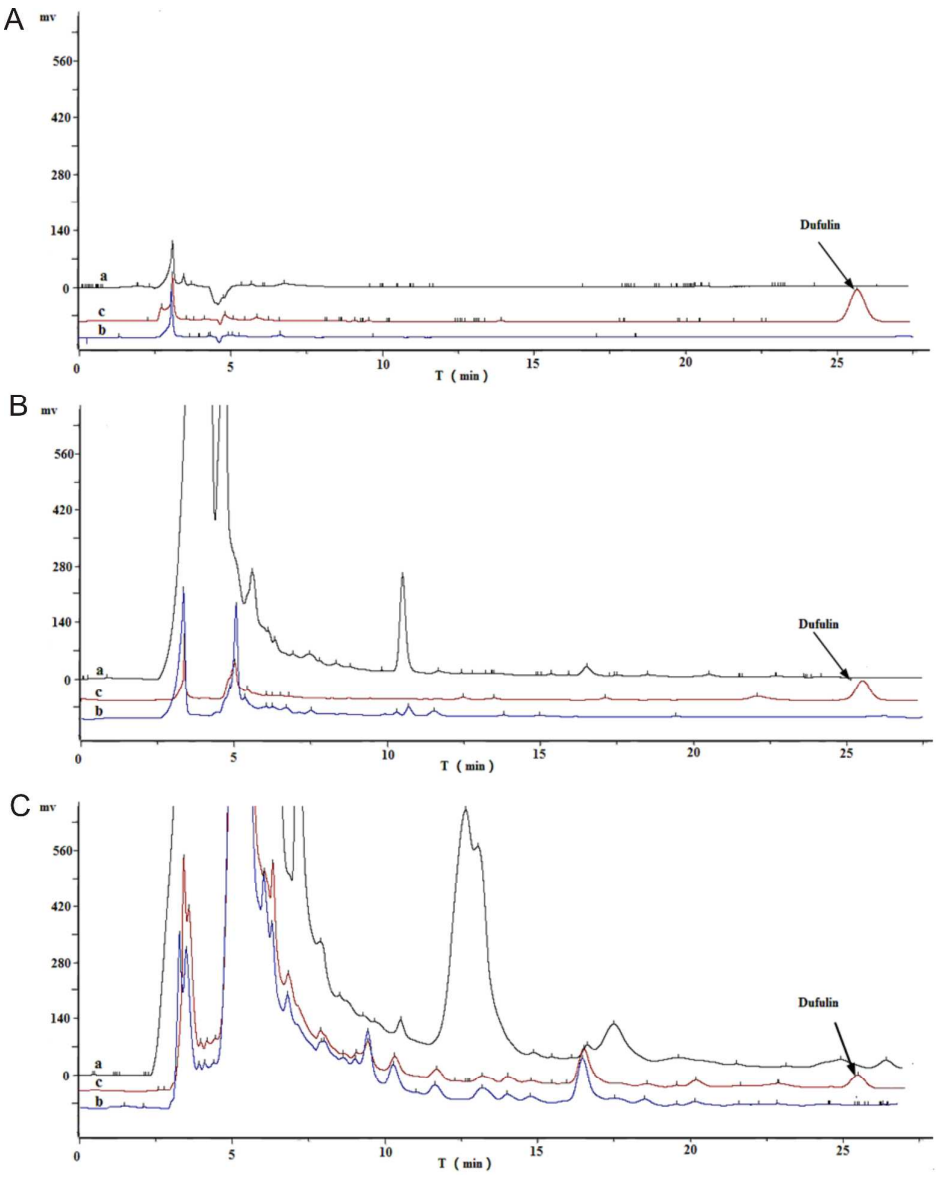


Fig. 5. Chromatograms of spiked Dufulin (0.5 mg $L^{-1}$) in water (A), soil (B) and wheat (C) samples: blank sample by direct injection (a); blank sample with MIP-SPE (b); spiked sample with MIP-SPE (c).