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Simultaneous determination of non-steroidal anti-inflammatory drugs in river water samples by liquid chromatography–tandem mass spectrometry using molecularly imprinted polymers as a pretreatment column

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ABSTRACT

A restricted access media-molecularly imprinted polymer (RAM-MIP) for flufenamic acid has been developed for the simultaneous determination of non-steroidal anti-inflammatory drugs (NSAIDs) in river water samples. The RAM-MIP was prepared using 4-vinylpyridine and ethylene glycol dimethacrylate as a functional monomer and cross-linker, respectively, by a multi-step swelling and polymerization method followed by a surface modification technique. The RAM-MIP for flufenamic acid showed excellent molecular recognition abilities for flufenamic acid and mefenamic acid, and moderate molecular recognition abilities for indomethacin, etodolac and ketoprofen. The simultaneous determination of NSAIDs (mefenamic acid, indomethacin, etodolac and ketoprofen) in river water samples was carried out by LC–MS/MS using the RAM-MIP for flufenamic acid as a pretreatment column. The concentrations of mefenamic acid, indomethacin and etodolac in river water samples were determined to be 0.4, 0.7 and 0.3 ng/L, respectively, while ketoprofen was below the limit of quantitation.

1. Introduction

The presence of active pharmaceuticals and metabolites in the aquatic environment has become a major concern in environment pollution [1]. This could be due to excretion of pharmaceuticals and their metabolites in urine and feces, and inappropriate disposal of unused pharmaceuticals [2]. Multiple classes of pharmaceuticals such as antiviral drugs, steroids and related hormones, β-blockers, non-steroidal anti-inflammatory drugs (NSAIDs) and antiepileptics are detected in waste, surface, ground and drinking water [1–3]. Among them, NSAIDs are widely used for human health care and most frequently detected in aquatic environments [4].

For the assays of NSAIDs in aquatic environments, solid-phase extraction (SPE) or solid-phase microextraction of environmental water samples was followed by gas chromatography-mass spectrometry combined with derivatization of these drugs, liquid chromatography–mass spectrometry or liquid chromatography–tandem mass spectrometry (LC–MS/MS) [4–9]. Furthermore, capillary electrophoresis combined with MS or photo diode-array detection followed SPE and on-line pre-concentration of environmental water samples [5,10]. SPE, where silica- and polymer-based materials are employed, played an important role in their ultra-trace analysis in order to concentrate NSAIDs and to remove interferences in environmental water samples [5]. In addition to those SPE sorbents, molecularly imprinted polymers (MIPs) could be used for extraction of NSAIDs in environmental water samples as compound- or group-selective sorbents [11–13]. However, since the molecularly imprinted SPE method so far employed was in an off-line mode, it was not so reproducible.

In our previous paper [14], we prepared the restricted access media (RAM)-MIP for cyclobarbital, which has characteristics of both RAM and MIP, by a multi-step swelling and polymerization method followed by a hydrophilic surface modification technique. The RAM-MIP could exclude water-soluble oligomers of humic materials and selectively recognize a target compound (cyclobarbital) and a group of compounds (antiepileptics). Therefore, the RAM-MIP was utilized for selective on-line extraction of ultra-trace amounts of antiepileptics in river water samples, followed by LC–MS/MS analysis.

In this study, the RAM-MIP for flufenamic acid, which is one of NSAIDs, was prepared and its molecular recognition abilities for NSAIDs were evaluated by LC. Furthermore, the simultaneous determination of NSAIDs (mefenamic acid, indomethacin, etodolac and ketoprofen) in river water samples was carried out by LC–MS/MS using the RAM-MIP for flufenamic acid as a pretreatment column.

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2. Experimental

2.1. Materials

Ethylene glycol dimethacrylate (EDMA), 2-vinylpyridine (2-VPY) and 4-vinylpyridine (4-VPY) were purchased from Tokyo Chemical Industry (Tokyo, Japan). Glycerol monomethacrylate (GMMA) and glycerol dimethacrylate (GDMA) were gifts from Fuso Chemical (Osaka, Japan). Polyvinyl alcohol (degree of polymerization = 500, saponification value = 86.5–89 mol%) and potassium peroxodisulfate were purchased from Nacalai Tesque (Kyoto, Japan). 2,2′-Azobis(2,4-dimethyl valeronitrile) (ADVN), methacrylic acid (MAA), flufenamic acid, mefenamic acid, indomethacin, ketoprofen and mefenamic acid-d_3 used as an internal standard (IS) were purchased from Wako Pure Chemical Industries (Osaka, Japan). Etodolac was purchased from Sigma–Aldrich Japan (Tokyo, Japan). The structures of NSAIDs used in this study are illustrated in Fig. 1. Water, acetonitrile and methanol of an LC–MS grade were obtained from Wako Pure Chemical Industries. Other reagents and solvents of an analytical-reagent grade were used without further purification. Water purified with a Purelab Ultra (Organo, Tokyo, Japan) was used to prepare eluents and sample solutions.

2.2. Preparation of MIPs and RAM-MIP

MIPs were prepared by a multi-step swelling and polymerization method, and RAM-MIP was prepared by a multi-step swelling and polymerization method followed by a hydrophilic surface modification technique as reported previously [15,16]. Similarly, non-imprinted polymers (NIPs) and RAM-NIP were prepared for comparison.

A water dispersion of uniformly sized polystyrene-seed particles (0.497 g/mL) was admixed with a microemulsion prepared from dibutyl phthalate as an activating solvent, 0.02 g of sodium dodecyl sulfate and 10 mL of water by sonication. For the preparation of MIPs and NIPs of *ca.* 5.5 μm in a particle diameter, a water dispersion of seed particles and dibutyl phthalate used were 0.085 and 0.24 mL, respectively, while for RAM-MIP and RAM-NIP of *ca.* 9 μm in a particle diameter, those were 0.02 and 0.048 mL, respectively.

This first-step swelling was carried out at room temperature for 15 h with stirring at 125 rpm until oil microdrops completely disappeared. A dispersion of 0.1875 g of ADVN as an initiator, 2.5 mL of toluene as a porogen, 10 mL of 4.8% polyvinyl alcohol aqueous solution as a dispersion stabilizer, and 12.5 mL of water was added to the dispersion of swollen particles. This second-step swelling was carried out at room temperature for 2 h with stirring at 125 rpm. A dispersion of flufenamic acid as a template molecule, 5 mL of EDMA as a cross-linker, MAA, 2-VPY or 4-VPY as a functional monomer, 10 mL of 4.8% polyvinyl alcohol aqueous solution and 12.5 mL of water was added to the dispersion of swollen particles. This third-step swelling was carried out at room temperature for 2 h with stirring at 125 rpm. After the third-step swelling was completed, the polymerization procedure was started at 50 °C under argon atmosphere with stirring at 160 rpm for 24 h. For the preparation of RAM-MIP and RAM-NIP, the polymerization procedure was carried out at 50 °C for 4 h and then hydrophilic monomers, 0.64 mL of GMMA and 0.64 mL of GDMA, together with 0.0256 g of potassium peroxodisulfate were added to the polymerizing materials. Further polymerization was carried out at 70 °C for 20 h. MIPs, RAM-MIP, NIPs and RAM-NIP prepared were shown in Table 1. After polymerization, a dispersion of polymerized particles was poured into 200 mL of methanol and the supernatant was discarded after sedimentation of the particles. The polymer particles were redispersed into methanol, and this procedure was repeated three times in methanol, once in water and twice in tetrahydrofuran. The resulting polymer particles were collected using a glass filter, washed with tetrahydrofuran and dried at room temperature.

2.3. Evaluation of MIP and RAM-MIP

The prepared materials were packed into a stainless steel column (100 mm × 4.6 mm ID or 10 mm × 4.0 mm ID) by a slurry packing technique using methanol–2-propanol (2:1, v/v) as a slurry solvent and methanol as a packing solvent to evaluate their chromatographic characteristics.

The LC system used was composed of a LC-10ADvp pump, a SPD-10Avp spectrophotometer, a C-R6A integrator (all from Shimadzu, Kyoto, Japan), a Rheodyne 7125 injector with a 20 μL loop (Rheodyne, Cotati, CA, USA), and a CO-1093 C column oven (Uniflows, Tokyo, Japan). The LC conditions used are specified in the legends of tables and figures.

The retention factor (k) was calculated from the equation $k = (t_R - t_0)/t_0$, where t_R and t_0 are the retention times of retained and unretained solutes, respectively. The retention time of an unretained solute, t_0, was measured by injecting the solution whose organic modifier content was slightly different from that of the eluent. The selectivity factor (S) was calculated from the equation $S = k_{MIP}/k_{NIP}$, where k_{MIP} and k_{NIP} are the retention factors of a solute on MIP and NIP, respectively. The selectivity factor was used to evaluate molecular recognition abilities of MIPs and RAM-MIP.

2.4. Column-switching LC–MS/MS conditions for simultaneous determination of NSAIDs in river water samples

A pretreatment system was composed of an 880-PU pump (Jasco, Tokyo, Japan) and a six-port switching valve (Analchem, Luton, UK). An alliance HT Waters 2795 separation module (Waters, Milford, MA, USA) and a Quattro premier triple–quadrupole mass spectrometer (Micromass, Manchester, UK) were used for LC–MS/MS analyses. Operation of the quaternary pump, mass spectrometer and data acquisition were controlled using Mass Lynx 4.1 software (Waters). A pretreatment column packed with RAM-MIP (10 mm × 4.0 mm ID) was equilibrated with 2 mM formic acid–acetonitrile (90:10, v/v) and 50 mL of a river water sample including 2 mM formic acid and 15% methanol was loaded.

Table 1
Template molecule and functional monomer used for the preparation of MIPs and RAM-MIP in this study.

Polymer	Template molecule		Functional monomer		Hydrophilic monomer
	Type	Amount (mmol)	Type	Amount (mmol)	
MIP1	Flufenamic acid	4	MAA	6	–
NIP1	–	–	MAA	6	–
MIP2	Flufenamic acid	4	2-VPY	6	–
NIP2	–	–	2-VPY	6	–
MIP3	Flufenamic acid	4	4-VPY	6	–
NIP3	–	–	4-VPY	6	–
RAM-MIP4	Flufenamic acid	4	4-VPY	6	GMMA/GDMA
RAM-NIP4	–	–	4-VPY	6	GMMA/GDMA

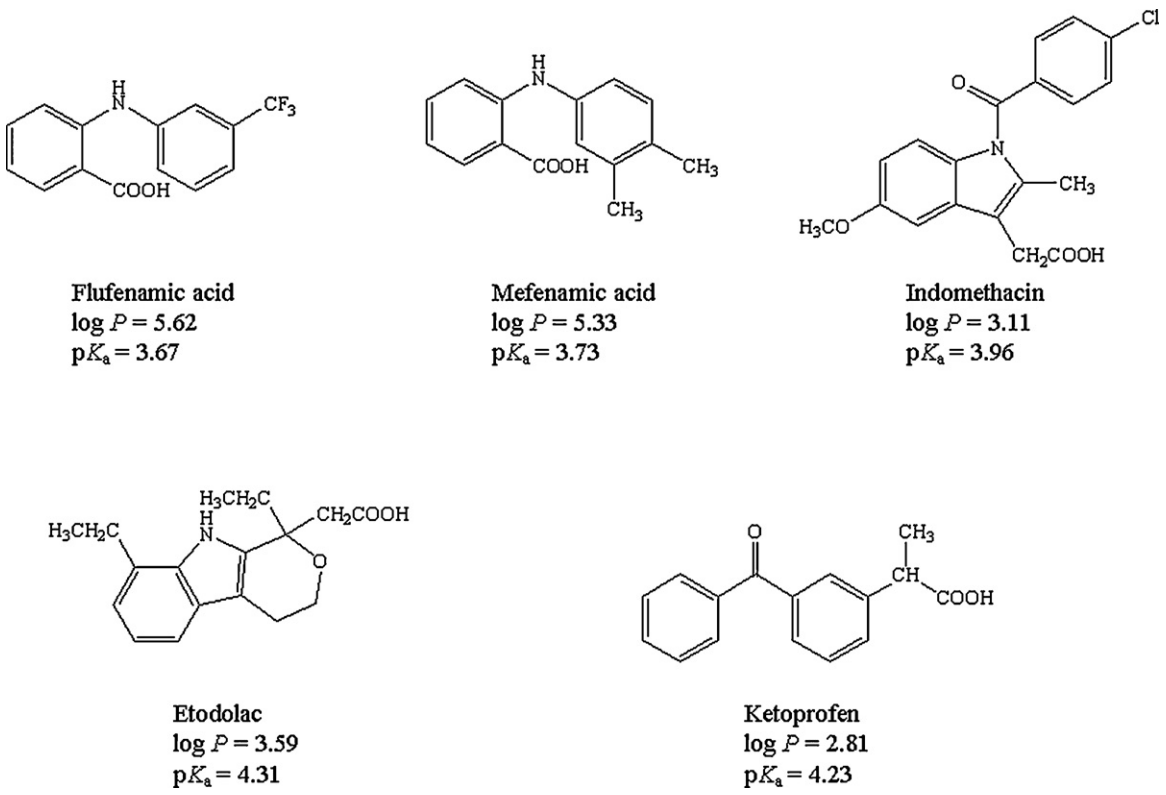

Fig. 1. Structures of NSAIDs used in this study.

For comparison, a pretreatment column packed with Cosmosil $5C_{18}$-MS (10 mm × 4.6 mm ID) was used instead of the RAM-MIP column. Next, the pretreatment column was washed for 12.5 min with 2 mM formic acid–acetonitrile (90:10, v/v) at a flow rate of 2.0 mL/min. Then, NSAIDs and IS retained on the pretreatment column were transferred to an analytical column [Cosmosil $5C_{18}$-MS-II (150 mm × 2.0 mm ID) and Cosmosil $5C_{18}$-MS-II guard column (10 mm × 4.6 mm ID), both from Nacalai Tesque] in the back-flush mode using 2 mM formic acid–acetonitrile (45:55, v/v) at a flow rate of 0.2 mL/min. The pretreatment and analytical columns were kept at 25 °C. The MS/MS conditions were as follows: ionization, positive ion mode electrospray; data collection, selected reaction monitoring (SRM) (mefenamic acid-d_3 (IS), m/z 245→227; indomethacin, m/z 358→139; etodolac, m/z 288→172; ketoprofen, m/z 255→105; mefenamic acid, m/z 242→180); source temperature, 100 °C; desolvation temperature, 350 °C; flow rate of corn nitrogen, 50 L/h; and flow rate of desolvation nitrogen, 800 L/h. Capillary and cone voltages are 3.0 kV and 20 V, respectively, and collision energy is 13 eV.

2.5. Method validation and simultaneous determination of NSAIDs in river water samples

River water samples were obtained from Naruo-shin river (Nishinomiya, Hyogo, Japan). The samples were stored at 4 °C and filtered through a 0.45 μm membrane filter prior to use. The river water samples were spiked with six different amounts of mefenamic acid, indomethacin, etodolac and ketoprofen, resulting in a final concentration of 0.3, 0.6, 1.5, 3.0, 6.0 and 12 ng/L as mefenamic acid and indomethacin, 0.2, 0.4, 1.0, 2.0, 4.0 and 8.0 ng/L as etodolac and ketoprofen. Mefenamic acid-d_3 at a final concentration of 10 ng/L was used as IS. By addition of formic acid and methanol to the spiked river water samples, those final concentrations were 2 mM and 15%, respectively. The calibration graphs were constructed by plotting the peak area ratios of the analyte to IS against the spiked analyte concentrations. The intra- and inter-day precision and accuracy data were obtained with the assays of spiked river water samples.

3. Results and discussion

3.1. Preparation and evaluation of MIP and RAM-MIP

MIP1, MIP2 and MIP3 for flufenamic acid, whose particle sizes are *ca.* 5.5 μm, were prepared using MAA, 2-VPY and 4-VPY, respectively, as the functional monomers by a multi-step swelling and polymerization method. Furthermore, RAM-MIP4, whose particle size is *ca.* 9 μm, was prepared using 4-VPY as the functional monomer by a multi-step swelling and polymerization method followed by surface modification. Fig. 2 shows the effect of eluent pH on retention factors of flufenamic acid, mefenamic acid, indomethacin and benzene on MIP1, MIP2 and MIP3 and RAM-MIP4, where a mixture of 50 mM potassium phosphate buffer–acetonitrile (30:70, v/v) is used as the eluent. As shown in Fig. 2A, flufenamic acid, mefenamic acid and indomethacin were not retained on MIP1, which is prepared by using an acidic functional monomer, MAA. On the other hand, these NSAIDs were retained on MIP2 and MIP3, which are prepared by using basic 2-VPY and 4-VPY, respectively, as the functional monomers, as shown in Fig. 2B and C. The retention factors of these NSAIDs on MIP2 and MIP3 were drastically decreased with an eluent pH >7. This could be due to dissociation of these NSAIDs, whose pK_a values are around 4.[1] The three MIPs seem to have similar hydrophobicity, judging from the retention factors of benzene. Thus, in addition to

[1] Calculated using Advanced Chemistry Development (ACD/Labs) Software V8.14 for Solaris (© 1994-2009 ACD/Labs).

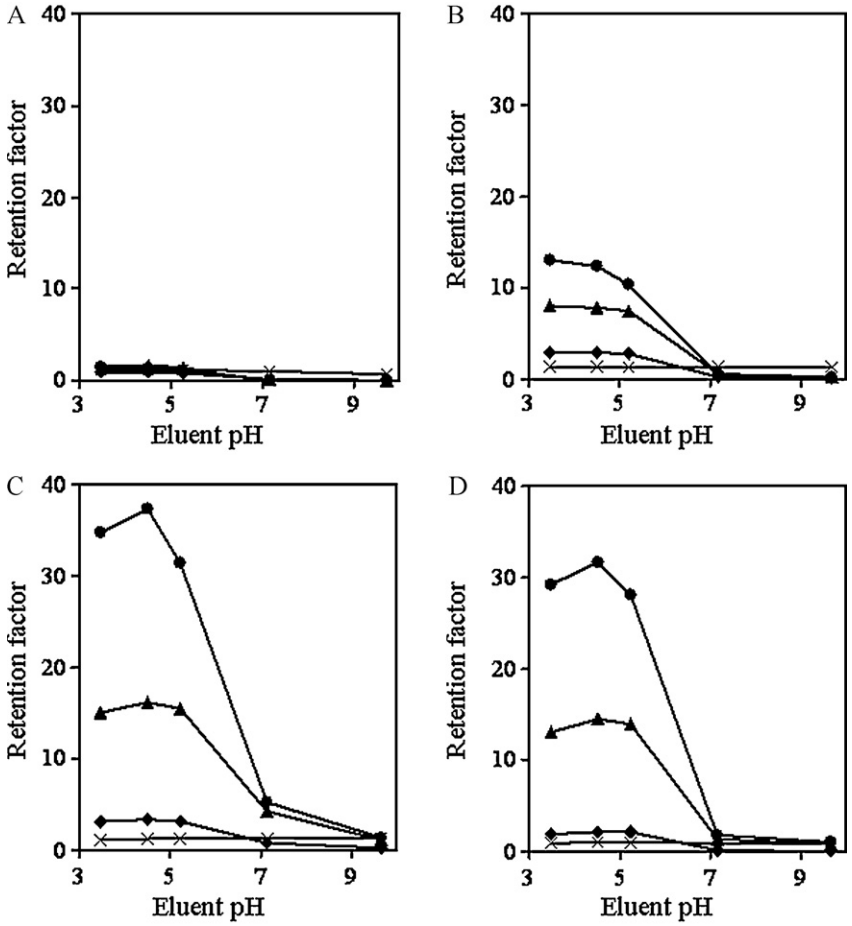

Fig. 2. Effect of eluent pH on the retention properties of flufenamic acid, mefenamic acid, indomethacin and benzene on MIP1 (A), MIP2 (B), MIP3 (C) and RAM-MIP4 (D). Keys: (●) flufenamic acid, (▲) mefenamic acid, (♦) indomethacin, (×) benzene. LC conditions: column size, 100 mm × 4.6 mm ID; column temperature, 25 °C; eluent, 50 mM potassium phosphate buffer–acetonitrile (30:70, v/v); detection, 210 nm; flow rate, 1.0 mL/min. loaded amount, 500 ng.

hydrophobic interactions, hydrogen bonding interactions between a pyridyl group of 2-VPY or 4-VPY and these NSAIDs could work for their retentions on both MIP2 and MIP3, as reported previously [17]. Furthermore, though flufenamic and mefenamic acid have similar log *P* values,[1] the former was retained more than the latter on MIP2 and MIP3 because of a molecular imprinting effect. These results indicate that in addition to shape recognition, hydrophobic and hydrogen bonding interactions play an important role in retentivity of these NSAIDs on MIP2 and MIP3. The pK_a values of 2-VPY and 4-VPY were calculated to be 4.95 and 5.39,[1] respectively. In a previous paper [17], it was reported that the average pK_a value of 4-VPY–EDMA copolymers shifted to <3. Similarly, it is assumed that the average pK_a value of 2-VPY–EDMA copolymers could shift to <3. On MIP2, the maximal retention factors of NSAIDs were not observed with eluent pH 4.5, but were observed on MIP3. These results suggest that at eluent pH *ca.* 3, the protonation of a pyridyl group could not occur on MIP2, but that could occur on MIP3. This could be due to differences in the average pK_a values of 2-VPY–EDMA and 4-VPY–EDMA copolymers.

RAM-MIP4 was prepared using the same feeding ratio of template molecule and functional monomer with MIP3. The retention tendencies of flufenamic acid, mefenamic acid and indomethacin on RAM-MIP4 were very similar to those on MIP3 except that these NSAIDs were less retained on RAM-MIP4, as shown in Fig. 2D. This could be ascribable to lower hydrophobicity of RAM-MIP4, compared to that of MIP3.

Table 2 shows the retention and selectivity factors of flufenamic acid, mefenamic acid, indomethacin, etodolac and ketoprofen on MIPs and RAM-MIP at eluent pH 5.3. Among the three MIPs, MIP3 gave the highest retention and selectivity factors. In addition to shape recognition, hydrophobic and hydrogen bonding interactions between 4-VPY–EDMA copolymers and these NSAIDs could work for their retention and recognition on MIP3. RAM-MIP4, whose outer surfaces are covered with hydrophilic polymers, gave better selectivity factors for these NSAIDs than MIP3, despite their lower retentions. This could be due to a decrease of non-specific interactions (hydrophobic interactions) in RAM-MIP4. These results are in good agreement with our previous results that the RAM-MIP for cyclobarbital gave higher selectivity and lower retentivity than the corresponding MIP [14].

In our previous study [16], the RAM-MIP for irgarol was applied for the determination of methyltriazine herbicides in river water

Table 2
Retention and selectivity factors of NSAIDs on MIPs and RAM-MIP.[a]

Solute	MIP1		MIP2		MIP3		RAM-MIP4	
	k	*S*[b]	*k*	*S*[b]	*k*	*S*[b]	*k*	*S*[b]
Fulfenamic acid	1.11	1.38	10.4	3.16	31.5	7.64	28.1	10.5
Mefenamic acid	1.39	1.26	7.41	2.02	15.5	3.32	14.0	5.09
Indomethacin	0.83	1.28	2.79	1.62	3.15	1.71	2.14	3.44
Etodolac	0.52	1.26	1.73	1.63	2.07	1.70	1.35	3.76
Ketoprofen	0.58	1.27	1.64	1.50	2.02	1.56	1.50	2.25

[a] LC conditions: column size, 100 mm × 4.6 mm ID; column temperature, 25 °C; eluent, 50 mM potassium phosphate buffer (pH 4.0)–acetonitrile (30:70, v/v, pH 5.3); flow rate, 1.0 mL/min; detection, 210 nm; loaded amount, 500 ng.
[b] *S* is the selectivity factor, k_{MIP}/k_{NIP}.

samples. It was shown that the RAM-MIP could more efficiently remove the interferences in river water samples than the MIP and be tolerable for repeated, larger volume loadings of river water samples. Furthermore, we used the RAM-MIP for cyclobarbital for removal of the interferences in river water samples and group-selective extraction of antiepileptics in river water samples [14]. In the following study, RAM-MIP4 was used as a pretreatment column for the simultaneous determination of NSAIDs in river water samples by LC-MS/MS.

3.2. Application of RAM-MIP for simultaneous determination of NSAIDs in river water samples

In our previous studies [14,16], river water samples were directly loaded onto RAM-MIPs for irganol and cyclobarbital without any pretreatments. However, direct injection of river water samples onto RAM-MIP4 resulted in almost no recovery of NSAIDs because river water samples were weakly basic. Dissociation of NSAIDs in river water samples interrupted their hydrophobic and hydrogen bonding interactions with RAM-MIP4. First, formic acid was added in order to acidify the river water samples. As shown in Fig. 3, the relative recoveries of NSAIDs were *ca.* 50% by addition of formic acid. Further addition of methanol to the acidified river water samples resulted in 90–100% recoveries of NSAIDs. In the following study, 2 mM formic acid and 15% methanol were added to river water samples.

We used a column-switching LC–MS/MS system. A 50 mL volume of a river water sample containing 2 mM formic acid and 15% methanol was loaded onto the RAM-MIP4 column using an LC pump at a flow rate of 4.0 mL/min for the determination of

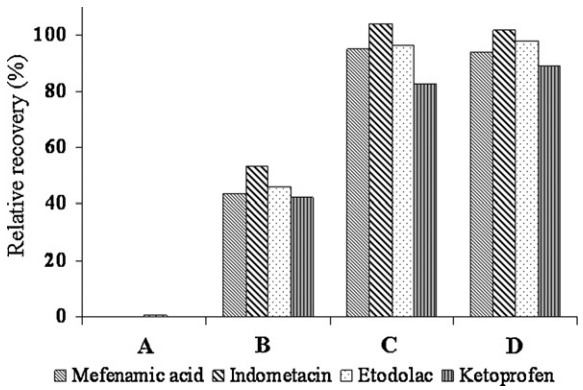

Fig. 3. Influence of addition of formic acid and methanol to a river water sample on relative recoveries of NSAIDs. A, no addition; B, addition of 2 mM formic acid; C, addition of 2 mM formic acid + 10% methanol; D, addition of 2 mM formic acid + 15% methanol.
Pretreatment conditions: column, RAM-MIP4 (10 mm × 4.0 mm ID); column temperature, 25 °C; injection volume, 50 mL (at 4.0 mL/min for 12.5 min) of river water samples; washing eluent, 2 mM formic acid–acetonitrile (90:10, v/v, 2.0 mL/min for 10 min). Analysis conditions: column, Cosmosil $5C_{18}$-AR-II (150 mm × 2.0 mm ID) and Cosmosil $5C_{18}$-AR-II guard column (10 mm × 4.6 mm ID); eluent, 2 mM formic acid–acetonitrile (45:55, v/v); flow rate, 0.2 mL/min; column temperature, 25 °C. MS/MS conditions: ionization, positive ion mode electrospray; data collection, SRM.

NSAIDs at ng/L level. In order to remove the interferences in river water samples, the RAM-MIP4 column was washed with 2 mM formic acid–acetonitrile (90:10, v/v). Fig. 4, parts A and B, shows SRM chromatograms of NSAIDs in river water samples obtained with conventional C18 and RAM-MIP4 columns, respectively, as

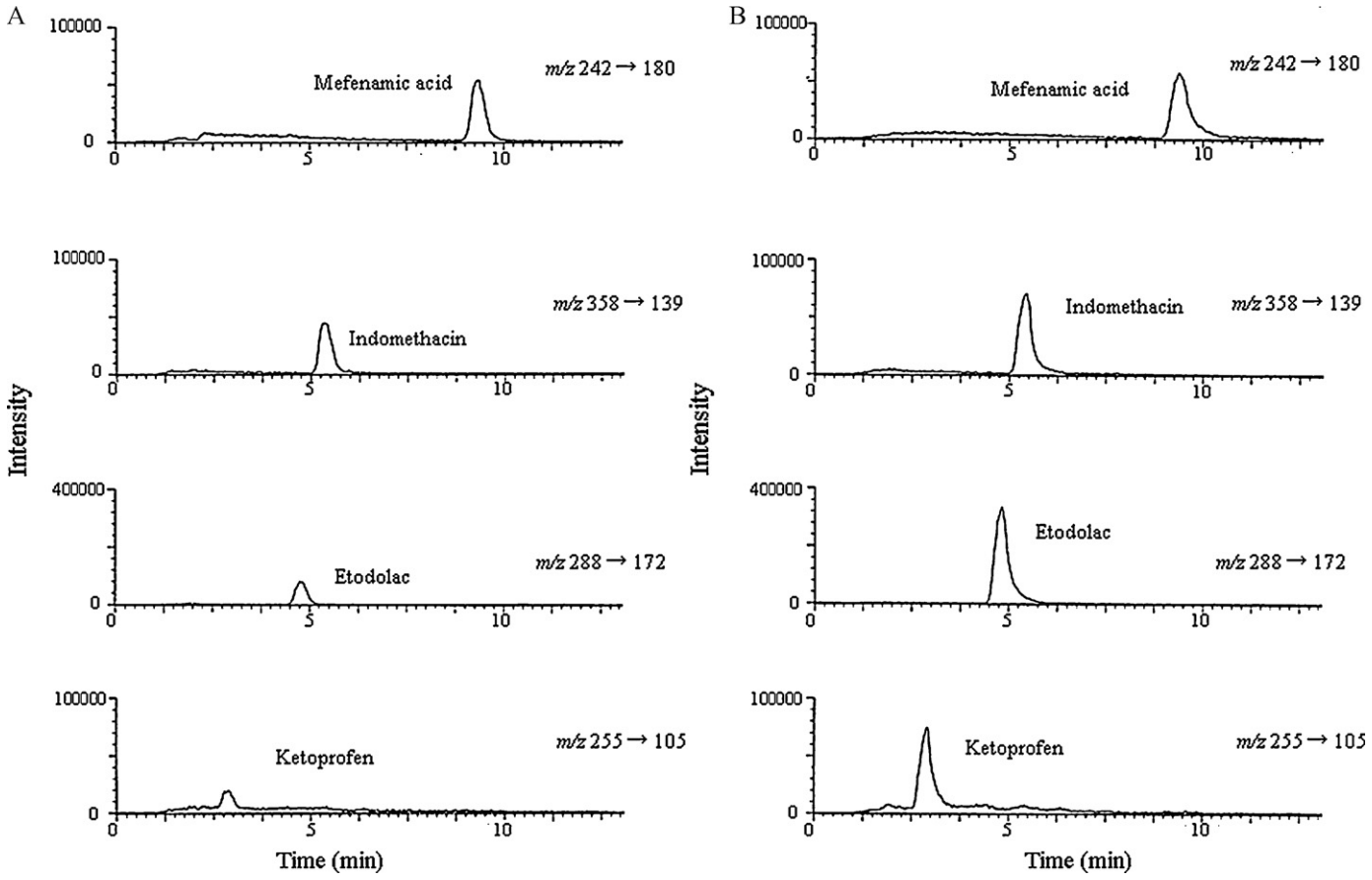

Fig. 4. SRM chromatograms of NSAIDs in river water samples obtained with C18 (A) or RAM-MIP4 (B) columns as the pretreatment columns. Pretreatment conditions as in Fig. 3 except for a pretreatment column, Cosmosil $5C_{18}$-AR-II (10 mm × 4.6 mm ID) or RAM-MIP4 (10 mm × 4.0 mm ID). Analysis conditions as in Fig. 3.

Table 3
Intra- and inter-day precision and accuracy data for the assays of mefenamic acid, indomethacin, etodolac and ketoprofen in spiked river water samples.

NSAIDs	Intra-day (n = 5)			Inter-day (n = 3)		
	Concentration (ng/L) added (measured)			Concentration (ng/L) added (measured)		
Mefenamic acid	0.3 (0.33)	1.5 (1.47)	6.0 (6.29)	0.3 (0.34)	1.5 (1.48)	6.0 (6.11)
Indomethacin	0.3 (0.27)	1.5 (1.48)	6.0 (6.51)	0.3 (0.26)	1.5 (1.46)	6.0 (6.42)
Etodolac	0.2 (0.19)	1.0 (0.97)	4.0 (4.14)	0.2 (0.17)	1.0 (0.96)	4.0 (4.05)
Ketoprofen	0.2 (0.19)	1.0 (1.03)	4.0 (3.92)	0.2 (0.20)	1.0 (0.99)	4.0 (3.97)

NSAIDs	Intra-day (n = 5)			Inter-day (n = 3)		
	RSD (%)[a]			RSD (%)[a]		
Mefenamic acid	2.78	3.50	2.33	0.56	1.34	3.67
Indomethacin	1.89	4.67	1.41	1.40	3.19	3.39
Etodolac	2.83	4.52	1.36	2.60	0.81	1.66
Ketoprofen	3.09	6.82	4.58	0.78	2.38	1.03

NSAIDs	Intra-day (n = 5)			Inter-day (n = 3)		
	Accuracy (% deviation)[b]			Accuracy (% deviation)[b]		
Mefenamic acid	11.2	−2.03	4.80	12.6	−1.43	1.90
Indomethacin	−8.91	−1.54	8.48	−14.0	−2.67	6.96
Etodolac	−7.44	−3.46	3.46	−14.8	−4.40	1.37
Ketoprofen	−3.42	2.89	−1.92	−1.59	−0.53	−0.63

[a] Relative standard deviation.
[b] % deviation = [(concentration measured − concentration added)/concentration added] × 100.

the pretreatment columns. When the C18 column was used for a pretreatment column, recoveries of indomethacin, etodolac and ketoprofen were low because of their partial elution from the column during loading of river water samples and/or washing the column with 2 mM formic acid–acetonitrile (90:10, v/v). On the other hand, RAM-MIP4 being used for a pretreatment column, recoveries of mefenamic acid, indomethacin, etodolac and ketoprofen were complete. These results clearly indicate that the RAM-MIP for flufenamic acid is useful for selective on-line extraction of ultra-trace amounts of non-steroidal anti-inflammatory drugs (NSAIDs) in river water samples. The RAM-MIP column was tolerable for at least 20-times injections of 50 mL of river water samples.

3.3. Method validation and simultaneous determination of NSAIDs in river water samples

Table 3 shows the precision and accuracy data of intra- and inter-day assays for the simultaneous determination of mefenamic acid, indomethacin, etodolac and ketoprofen in river water samples. This method was accurate and reproducible as shown in Table 3. The calibration graphs, constructed from peak area ratio of the analyte (mefenamic acid, indomethacin, etodolac and ketoprofen) to mefenamic acid-d_3 (IS) versus the spiked analyte concentration, were linear with a correlation coefficient of >0.999 with a 50 mL injection of river water samples. The equations were $y = 0.151,x + 0.005$ for mefenamic acid in the concentration ranges of 0.3–12 ng/L, $y = 0.196,x + 0.014$ for indomethacin in the concentration ranges of 0.3–12 ng/L, $y = 0.838,x + 0.130$ for etodolac in the concentration ranges of 0.2–8.0 ng/L, and $y = 0.123,x + 0.005$ for indomethacin in the concentration ranges of 0.2–8.0 ng/L, where x is the analyte concentration and y is the peak area ratio. The limits of quantitation for mefenamic acid, indomethacin, etodolac and ketoprofen were 0.3, 0.3, 0.2 and 0.2 ng/L, respectively, and the limits of detection were 0.15, 0.15, 0.1 and 0.1 ng/L, respectively, at a signal to noise ratio of 3.

Fig. 5 shows SRM chromatograms of river water samples by a column-switching LC–MS/MS system. The concentrations of mefenamic acid, indomethacin and etodolac in river water samples were determined to be 0.4, 0.7 and 0.3 ng/L, respectively, while ketoprofen was below the limit of quantitation.

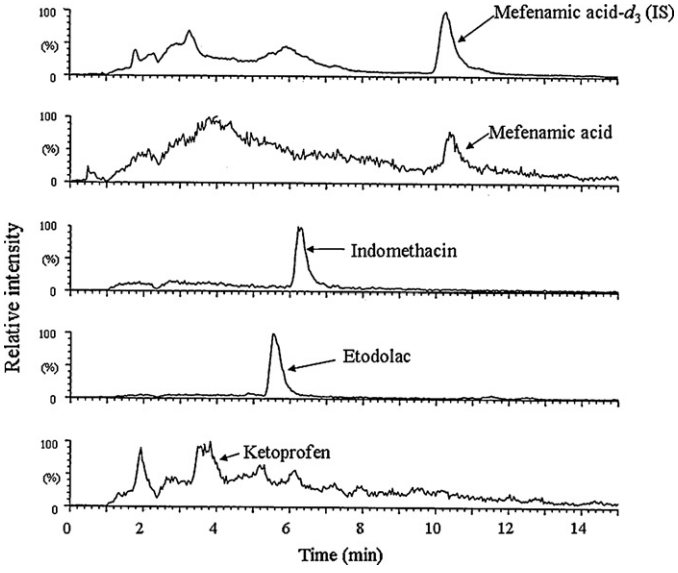

Fig. 5. SRM chromatograms of river water samples obtained with a column-switching LC–MS/MS system with RAM-MIP4 as a pretreatment column. Pretreatment conditions and analysis conditions as in Fig. 3.

4. Conclusions

We prepared a RAM-MIP for flufenamic acid by a multi-step swelling and polymerization method followed by a hydrophilic surface modification technique. The RAM-MIP for flufenamic acid showed molecular recognition abilities for mefenamic acid, indomethacin, etodolac and ketoprofen as well as flufenamic acid. The simultaneous determination of NSAIDs (mefenamic acid, indomethacin, etodolac and ketoprofen) in river water samples was carried out by LC–MS/MS using the RAM-MIP for flufenamic acid as a pretreatment column. The concentrations of mefenamic acid, indomethacin and etodolac in river water samples

were determined to be 0.4, 0.7 and 0.3 ng/L, respectively, while ketoprofen was below the limit of quantitation.

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References

[1] A. Nikolaou, S. Meric, D. Fatta, Occurrence patterns of pharmaceuticals in water and wastewater environments, Anal. Bioanal. Chem. 387 (2007) 1225–1234.
[2] J. Fick, H. Söderström, R.H. Lindberg, C. Phan, M. Tysklind, D.G.J. Larsson, Contamination of surface, ground, and drinking water from pharmaceutical production, Environ. Toxicol. Chem. 28 (2009) 2522–2527.
[3] C. Richman, S. Castensson, Impact of waste pharmaceuticals: an environmental hazard or "greenwash", Pharm. J. 280 (2008) 335–342.
[4] M. Farré, M. Petrovic, M. Gros, T. Kosjek, E. Martinez, E. Heath, P. Osvald, R. Loos, K. Le Menach, H. Budzinski, F. De Alencastro, J. Müller, T. Knepper, G. Fink, T.A. Ternes, E. Zuccato, P. Kormali, O. Gans, R. Rodil, J.B. Quintana, F. Pastori, A. Gentili, D. Barceló, First interlaboratory exercise on non-steroidal anti-inflammatory drugs analysis in environmental samples, Talanta 76 (2008) 580–590.
[5] A. Gentili, Determination of non-steroidal anti-inflammatory drugs in environmental samples by chromatographic and electrophoretic techniques, Anal. Bioanal. Chem. 387 (2007) 1185–1202.
[6] S. Marchese, A. Gentili, D. Perret, G. D'Ascenzo, F. Pastori, Quadrupole time-of-flight versus triple–quadrupole mass spectrometry for the determination of non-steroidal antiinflammatory drugs in surface water by liquid chromatography/tandem mass spectrometry, Rapid Commun. Mass Spectrom. 17 (2003) 879–886.
[7] S. Marchese, D. Perret, A. Gentili, R. Curini, F. Pastori, Determination of non-steroidal anti-inflammatory drugs in surface water and wastewater by liquid chromatography–tandem mass spectrometry, Chromatographia 58 (2003) 263–269.
[8] J. Dębska, A. Kot-Wasik, J. Namieśnik, Determination of nonsteroidal anti-inflammatory drugs in water samples using liquid chromatography coupled with diode-array detector and mass spectrometry, J. Sep. Sci. 28 (2005) 2419–2426.
[9] A. Kot-Wasik, J. Dębska, A. Wasik, J. Namieśnik, Determination of non-steroidal anti-inflammatory drugs in natural waters using off-line and on-line SPE followed by LC coupled with DAD-MS, Chromatographia 64 (2006) 13–21.
[10] M. Dawod, M.C. Breadmore, R.M. Guijt, P.R. Haddad, Electrokinetic supercharging for on-line preconcentration of seven non-steroidal anti-inflammatory drugs in water samples, J. Chromatogr. A 1189 (2008) 278–284.
[11] T. Yang, Y.-H. Li, S. Wei, Y. Li, A. Deng, Development of a selective molecularly imprinted polymer-based solid-phase extraction for indomethacin from water samples, Anal. Bioanal. Chem. 391 (2008) 2905–2914.
[12] S. Zorita, B. Boyd, S. Jonsson, E. Yilmaz, C. Svensson, L. Mathiasson, S. Bergstrom, Selective determination of acidic pharmaceuticals in wastewater using molecularly imprinted solid-phase extraction, Anal. Chim. Acta 626 (2008) 147–154.
[13] E. Caro, R.M. Marcé, P.A.G. Cormack, D.C. Sherrington, F. Borrull, Selective enrichment of anti-inflammatory drugs from river water samples by solid-phase extraction with a molecularly imprinted polymer, J. Sep. Sci. 28 (2005) 2080–2085.
[14] K. Hoshina, S. Horiyama, H. Matsunaga, J. Haginaka, Molecularly imprinted polymers for simultaneous determination of antiepileptics in river water samples by liquid chromatography–tandem mass spectrometry, J. Chromatogr. A 1216 (2009) 4957–4962.
[15] H. Sambe, K. Hoshina, K. Hosoya, J. Haginaka, Simultaneous determination of bisphenol A and its halogenated derivatives in river water by combination of isotope imprinting and liquid chromatography–mass spectrometry, J. Chromatogr. A 1134 (2006) 16–23.
[16] H. Sambe, K. Hoshina, J. Haginaka, Molecularly imprinted polymers for triazine herbicides prepared by multi-step swelling and polymerization method. Their application to the determination of methylthiotriazine herbicides in river water, J. Chromatogr. A 1152 (2007) 130–137.
[17] J. Haginaka, H. Sanbe, H. Takehira, Uniform-sized molecularly imprinted polymer for (*S*)-ibuprofen. Retention properties in aqueous mobile phases, J. Chromatogr. A 857 (1999) 117–125.