Effective determination of a pharmaceutical, sulpiride, in river water by online SPE-LC-MS using a molecularly imprinted polymer as a preconcentration medium

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22 **Abstract**

23 We report an effective and a quantitative analysis method for one of pharmaceuticals, sulpiride, in
24 river water by online solid phase extraction (SPE) connected with liquid chromatography–mass
25 spectrometry (LC–MS) using a molecularly imprinted polymer as a preconcentration medium. The
26 polymer prepared with a pseudo template molecule showed the selective retention ability based on the
27 interval recognition of functional groups in sulpiride. Also, the imprinted polymer provided an effective
28 concentration of a trace level of sulpiride in offline SPE with dual washing processes using water and
29 acetonitrile, although another imprinted polymer prepared by an authentic method using sulpiride and
30 methacrylic acid as a template and a functional monomer, respectively, showed the selective adsorption
31 only in organic solvents. Furthermore, we employed the imprinted polymer as the preconcentration
32 column of online SPE–LC–MS and the results supposed that the proposed system allowed the
33 quantitative analysis of sulpiride with high sensitivity and recovery (10 ng/L at 96%). Additionally, the
34 determination of sulpiride in real river water without an additional spiking was effectively achieved by
35 the system.

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38 *Keywords:* molecular imprinting, interval recognition, solid phase extraction, pharmaceutical, sulpiride

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47 **1. Introduction**

48 Molecularly imprinted polymers (MIPs) have been widely developed for separations, sensors,
49 catalysis, and biological mimics based on the selective molecular recognition of small and/or large
50 molecules [1-7]. The concept of the MIP is simple and easy to obtain the selective molecular
51 recognition by polymerization of a self-assembled complex between a template (target molecule) and
52 functional monomers under excess of a porogenic solvent and crosslinker. In general, the polymers are
53 prepared with the complex interacted through non covalent bonding, such as hydrogen bonding,
54 hydrophobic interaction, and ionic bonding, in organic solvents. Therefore, an authentic method can be
55 easily applied for the selective separation and adsorption of relatively hydrophobic compounds.
56 Similarly, we previously reported the selective separations of environmental pollutants. However, few
57 studies have been reported for the selective recognition of highly hydrophilic compounds, such as water
58 soluble alkaloids and pharmaceutical products. To figure out the limitation in the authentic method, we
59 previously reported the new concept named “interval immobilization technique” [8, 9] which allows the
60 selective molecular recognition for the water soluble compounds including ionic groups by the
61 recognition of intervals between ionic groups on a targeting molecule. The concept provided the
62 effective separation and/or concentration of natural toxins, and then contributed the sensitive
63 determination of the toxins.

64 On the other hand, pharmaceuticals and personal care products (PPCPs) in our water and soil
65 environment are listed by the US Environmental Protection Agency (EPA), and paid attention of their
66 effects against the ecological and biological systems. Many of studies have been reported about PPCPs
67 to clarify the effects and analyses methods [10-15]. Some of papers referred that most of PPCPs are not
68 removed in sewage-disposal plant and infallen into our water environment [16-19]. As the first
69 requirement for the risk management regarding PPCPs, we must establish the simple and effective
70 analytical methods for each compounds. In general, the concentration of PPCPs in environment is pretty
71 low (less than 1.0 µg/L) and the larger amount of contaminants are co-existing, therefore the
72 pretreatment processes to concentrate the targeting compounds and remove the contaminants are

73 required before final analyses using liquid chromatography (LC) combined with a photo diode array
74 detector (PDA), fluorescent detector (FL), and mass spectrometer (MS). Nowadays, solid phase
75 extraction (SPE) is usually utilized for the pretreatment even for PPCPs analyses. According to the
76 established practice, the hydrophobic adsorbents such as octadecyl group bonded silica (ODS) and
77 styrene-divinylbenzene based polymer are utilized for SPE of PPCPs. However, the complicated
78 protocols to collect each fraction are needed and the comfortable concentrations for detection are not
79 achieved because most of PPCPs have relatively low hydrophobicity. Therefore, the final results of the
80 LC determination are affected by artificial errors in SPE and a tandem mass spectrometer (MS/MS) is
81 often employed for the sensitive detections. Consequently, the effective pretreatment procedure
82 including the selective concentration of each PPCPs is necessary, so that PPCPs will be detectable by
83 universal detectors such as a PDA, FL, and single MS.

84 In this paper, we describe the selective preconcentration of one of PPCPs, sulpiride, by an MIP
85 enabling the selective concentration based on the recognition toward the intervals of ionic groups in
86 sulpiride. Sulpiride is regularly utilized to domestic use especially for antidepressant drugs and often
87 detected in water environment at high concentration [20-26]. Therefore, we assume that the
88 establishment of a simple analysis method for sulpiride will be useful for monitoring as a marker of
89 PPCPs pollutions. Finally, the effective determination of sulpiride at a trace level and in real river water
90 is demonstrated by an online SEP–LC–MS using the MIP as a pretreatment column.

91

92 **2. Experimental**

93 *2.1. Chemicals and instruments*

94 Ethylene glycol dimethacrylate (EDMA) as a crosslinking agent was purchased from Wako
95 Chemicals (Osaka, Japan) and distilled under vacuum to remove the polymerization inhibitor.
96 Tributylamine, 2,2'-azobis-(2,4-dimethylvaleronitrile) (ADVN), potassium peroxodisulfonate, *p*-styrene
97 sulfonic acid sodium salt (SSA), methacrylic acid (MAA), sulpiride, the other solutes for HPLC, and

other solvents including chloroform, toluene, tetrahydrofuran (THF), high performance liquid chromatography (HPLC) grade methanol (MeOH), and HPLC grade acetonitrile (MeCN) were also purchased from Wako Chemicals. 4,4'-bis (chloromethyl) biphenyl, tetra-*n*-butylammonium chloride (TBA), and benzyl tributylammonium chloride (BTBA) were purchased from Tokyo Kasei (Tokyo, Japan).

LC and LC–MS data were acquired by an LC–MS system (Shimadzu Co., Kyoto, Japan) consisted of LC-10AD as a pump, SPD-M10A as a PDA detector, CTO-10AC as a column oven, LCMS-2010A as a MS detector, and LCMS solution as an operation system.

The chromatographic parameters are defined as follows;

Retention factor, $k = (t_R - t_0) / t_0$, t_R : retention time of solute, t_0 : retention time of acetone

Separation factor, $\alpha = k_T / k_S$, k_S : retention factor of S, k_T : retention factor of T

2.2. Synthesis of template and ionic complex

The synthesis of 4,4'-bis-(tributylammonium-methyl chloride) biphenyl (BTAB) was carried out according to our previous study [9], briefly as follows: 4,4'-bis (chloromethyl) biphenyl (1.0 g) and tri-*n*-butylamine (6.0 mL) were dissolved in MeCN (50 mL). The mixture was stirred at 100 °C for 24 h under N₂ with reflux. After the reaction, the product was purified by silica gel chromatography using chloroform as a solvent. The isolated compound was assigned by ¹H-NMR and FT-IR.

Both of BTAB (0.46 g) and SSA (0.85 g) were dissolved in water, and then the produced complex was extracted with ethyl acetate. After dehydration and removal of the solvent of ethyl acetate layer, the residual was dried in vacuum and the complex (SSA–BTAB–SSA) was obtained.

2.3. Preparation of MIP

To obtain an imprinted polymer by the interval immobilization technique (I-MIP), polymer based particles with EDMA as a crosslinker were prepared by multi-step swelling and polymerization method

123 [27-29]. The schematic image of the I-MIP preparation is described in Fig. 1. The EDMA based
124 polymer particles (5.25 g) were dispersed in methanol, and then SSA–BTAB–SSA (87.0 mg) with
125 potassium peroxodisulfonate in water was added to the methanol solution. The mixture was stirred at
126 105 °C for 24 h. After filtration with a 0.45 µm membrane filter, the polymer was washed with
127 methanol/1.0 M NaCL aq. (v/v) to remove the template molecules and unreacted reagents. Similarly, a
128 non-imprinted polymer (I-NIP) was prepared with TBA–SSA complex at the same mole amount against
129 SO_3^- groups of MIP.

130 Additionally, to compare the selective separation ability, another MIP prepared by an authentic
131 molecular imprinting with sulpiride as a template (sul-MIP), using MAA as a functional monomer and
132 EDMA as a crosslinker. Also, NIP was prepared without template. The detailed procedures are
133 described in Supplementary data.

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135 2.4. Chromatographic evaluations

136 The I-MIP, I-NIP, sul-MIP, and NIP were packed into a stainless steel column (100 mm × 4.6 mm
137 i.d.) and evaluated with a buffered solution /MeCN as a mobile phase using hexylbenzene, aniline, *N,N*-
138 dimethyl aniline (DMA), and sulpiride as the solutes.

139 In order to examine the adsorption capacity and recognition mechanism, the frontal analyses were
140 carried out using the I-MIP or I-NIP packed column (30 mm × 4.6 mm i.d.) with BTAB and BTBA (500
141 mg/L) as mobile phases.

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143 2.5. Offline SPE evaluation

144 Each I-MIP and I-NIP (100 mg) was packed into a polypropylene cartridge. Each cartridge was
145 conditioned with methanol, water, and then sulpiride aqueous solution (10 µg/L) (10 mL each) was
146 eluted. Water and MeCN were eluted (1.0 mL each) for washing, finally 100 mM formate buffer (pH

147 3.0) / acetonitrile = 2/8 (v/v) was eluted (1.0 mL) to collect adsorbed sulpiride. Each fraction was
148 analyzed by LC–MC to determine the concentration of sulpiride.

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150 2.6. Online SPE–LC–MS for determination of sulpiride in river water

151 To achieve the simple and effective determination for sulpiride, we employed the I-MIP column as a
152 pretreatment column of an online column switching SPE–LC–MS system. We previously clarified the
153 advantages of the system for determination of trace leveled environmental pollutions.[30, 31] In this
154 study, the determination of sulpiride was operated by the online system employed the I-MIP. At first, to
155 evaluate the accuracy of the system, a sulpiride standard solution (10 ng/L, 50 ng/L, 100 ng/L in water)
156 was analyzed. Secondly, a real river water sample without spiking sulpiride was analyzed and the
157 concentration of sulpiride was determined. Here, the river water was diluted to 10 times with water
158 because the concentration of sulpiride was too high to detect by the online SPE-LC-MS. The river water
159 was collected from *Nishitenjin river* (Kyoto city) which is situated downstream of a sewage-disposal
160 plant. The river water sample was stored at 4 °C and filtrated with a 0.2 µm membrane filter before
161 using as a sample.

162

163 3. Results and discussion

164 3.1. Selectivity for sulpiride in the prepared polymers

165 To examine the selective recognition ability of each polymer prepared, the columns packed with the
166 I-NIP, I-MIP or sul-NIP, MIP were evaluated by LC. At first, the NIP and sul-MIP were evaluated with
167 buffer/MeCN as mobile phases using sulpiride, hexylbenzene, aniline, DMA, benzamide, and *N,N*-
168 dimethylbenzylamine (DMBA) as solutes. The retention factors of each solute are summarized in Fig.
169 S1 to S3. These results showed that the selectivity for sulpiride was not observed in buffer/MeCN as
170 mobile phases and the retention factors of solutes were almost the same in both NIP and sul-MIP.
171 Herein, the pK_a values of sulpiride are $pK_{a1}=9.00$ and $pK_{a2}=10.2$, hence sulpiride should dissociate in

aqueous solutions. Therefore, the hydrogen bonding and weakly ionic interaction by carboxylic acid of the sul-MIP were not workable for the selective recognition of sulpiride. In fact, as shown in Fig. S2, S3, the obvious selectivity for sulpiride was observed as increase of the MeCN content in the mobile phase. With 100% MeCN as a mobile phase, sulpiride was retained selectively in the sul-MIP, whereas the retentions of DMBA were almost the same in both columns. These results suggested that the hydrogen bonding was worked as the majority of the retention mechanism and imprinting effect was effectively performed. However, the authentic molecular imprinting is not suitable for the selective pretreatment of water soluble compounds such as pharmaceuticals in the environmental water sample because the selectivity is observed only in high content of organic solvents.

Secondly, the polymers prepared with the pore surface modification were evaluated by LC. The chromatograms on the I-MIP and I-NIP are shown in Fig. 2, and the separation factors for sulpiride are summarized in Fig. 3. Firstly, sulpiride was never eluted because of the strong adsorption by SO_3^- groups of the I-MIP and I-NIP in the mobile phases consisted of acetonitrile or water without a buffered solution. Therefore, to examine the selectivity of the columns, a formate buffer (pH 3.0) was employed. As shown in Fig. 2, sulpiride was selectively retained on the I-MIP, whereas the retention time of the other solutes were almost the same in both columns. Fig. 3 also showed the selective retention ability for sulpiride on the I-MIP. These results clearly suggested that the interval immobilization technique described in Fig. 1 provided the selective recognition for the targeting molecule.

3.2. Frontal analyses

To clarify the selectivity by the interval recognition of functional groups, the frontal analyses were carried out with the I-MIP and I-NIP columns. BTBA and BTAB, which are mono- and di-ammonium solutes, respectively, were utilized for the evaluations. The obtained chromatograms are shown in Fig. 4. According to the initial rising of BTBA peaks, we assumed that the ion-exchangeable capacity were almost the same in both columns. On the other hand, the initial rising of BTAB peaks was obviously

different in each other. Herein, if the ionic interaction is dependent on one to one between SO_3^- in polymer and a solute, the initial rising of BTAB should be observed at twice time as that of BTBA since mole amount of BTAB was half of BTBA. Actually, the time of initial rising of BTAB was almost twice as BTBA in the I-NIP. However, in the I-MIP, the time of BTAB was much earlier than twice time of BTBA. In other words, a number of SO_3^- groups in the I-MIP were consumed for “two-point” recognition of BTAB, therefore the adsorption of BTAB was much less than that of the I-NIP. From these results, we can estimate the ratio of “two-point” recognition against total SO_3^- groups at about 75%.

3.3. Adsorption of sulpiride by SPE

Since the selective adsorption of sulpiride by the interval recognition of functional groups was revealed in above discussion, the I-MIP was employed for an adsorbent of SPE. Before the SPE evaluations, we optimized the analysis conditions of sulpiride. To obtain the high sensitivity and resolution on LC, we choose hydrophilic interaction chromatography (HILIC) and MS detection. All conditions were optimized, resulting the high linearity and accuracy of calibration curve was obtained in the range from 10 to 1000 $\mu\text{g/L}$ ($R^2 = 0.9989$, $n = 3$). The limit of detection and limit of quantitation in 2.0 μL injection were estimated at 3.9 $\mu\text{g/L}$ ($S/N = 3$) and 13.1 $\mu\text{g/L}$ ($S/N = 10$), respectively.

Then, we optimized the protocols of the SPE evaluation for sulpiride by the I-MIP packed cartridge. As mentioned above, the selective adsorption of sulpiride was not observed in aqueous condition with the polymers prepared by an authentic molecular imprinting. Indeed, the sulpiride was eluted at first 1.0 mL fraction of passed through sample (10 $\mu\text{g/L}$, sulpiride in water) when the sul-MIP or NIP was utilized for SPE. Similarly, sulpiride was detected at first 3.0 mL fraction with the I-NIP. In contrast, when I-MIP was utilized for SPE, sulpiride was completely adsorbed to the I-MIP more than 25.0 mL elution of sample. Interestingly, the adsorbed sulpiride was still stable during water or MeCN washing. In brief, the I-MIP cartridge can be washed by both water and organic solvents after the sample

222 application. In this evaluation, 100 mM formate buffer (pH 3.0) / MeCN = 2/8 (v/v) was chosen for the
223 collection solvent which can be used for the LC–MS analysis below-mentioned. The obtained
224 chromatograms of each fraction are summarized in Fig. 5. As shown in these chromatograms, sulpiride
225 was not detected in the washing fraction and the recovery in the collected fraction (1.0 mL) was at
226 97.0%.

227

228 *3.4. Online SPE–LC–MS for determination of sulpiride*

229 As mentioned above, the I-MIP prepared by the interval immobilization technique provided the
230 selective retention for sulpiride and showed the capability for the effective concentration of sulpiride.
231 Finally, we demonstrate the practical usage of the I-MIP for the preconcentration of the diluted sulpiride
232 in the environmental water sample. Then, we employed online SPE–LC–MS described in Fig. 6. The
233 system was consisted of an analytical line connected with a pretreatment line through the valve. First, to
234 clarify the quantitative accuracy of the system, online analyses were carried out with a standard
235 sulpiride solution in water. The obtained chromatogram of sulpiride (10 ng/L) after concentration is
236 shown in Fig. 7. The chromatogram shows the effective concentration of sulpiride, and Table 1 indicates
237 the acceptable recoveries and relative standard deviations (RSD) in all the concentrations (10, 50, 100
238 ng/L). Then, the real river water sample **without spiking sulpiride** was analyzed by the same method. As
239 a result, the peak of sulpiride was clearly identified and determined at the concentration of 560 ng/L
240 with high accuracy (**RSD 4.3%, $n=3$**) (Fig. 8). These results supposed that the newly developed
241 SPE–LC–MS system using the I-MIP as a pretreatment is useful for the direct detection of
242 pharmaceuticals in the environmental water sample.

243

244 **4. Conclusion**

245 In this study, we developed the new determination system for one of PPCPs, sulpiride, by online
246 SPE–LC–MS using a polymer adsorbent. The polymer (I-MIP) prepared by the interval immobilization

247 technique based on the molecular imprinting showed the selective retention ability for sulpiride
248 according to the interval recognition of ionic groups. Also, we clarified the molecular recognition
249 mechanism in the I-MIP by frontal analyses and its stable retention ability in both water and organic
250 solvents. Furthermore, the possibility of practical application for the determination of sulpiride in the
251 environmental water sample by offline SPE and online SPE–LC–MS evaluations were demonstrated
252 with the high sensitivity, recovery, and accuracy. We expect that similar systems using several kinds of
253 MIPs will be applicable for the quantitative determinations of pharmaceutical products in the
254 environmental and biological samples.

255

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261 **Appendix A. Supplementary data**

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

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366 Table 1. Accuracy of analyses by online SPE–LC–MS for sulpiride

	Concentration of sulpiride		
	10 ng/L	50 ng/L	100 ng/L
RSD (%), $n = 3$	2.4	6.8	4.3
Recovery (%)	97.0	97.2	102

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368 Standard sulpiride was diluted to the certain concentration with water.

369 The SPE-LC-MS conditions were same as Fig.5.

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Figure captions

Fig. 1. Schematic images of the I-MIP for sulpiride.

393 Fig. 2. Chromatograms by the I-MIP or I-NIP packed columns.
394 LC Conditions: mobile phase, 50 mM formate buffer (pH 3.0)/MeCN = 2/8 (v/v); column size, 100 mm
395 × 4.6 mm i.d.; flow rate, 0.5 mL/min; detection, UV 254 nm; temperature, 40 °C; injection, 5 µL;
396 solutes, 1 acetone (1000 ppm), 2 aniline (100 ppm), 3 hexylbenzene (100 ppm)

397
398 Fig. 3. Separation factors against k of sulpiride on the I-MIP and I-NIP.

399 LC conditions were the same as Fig. 2. DMA, *N,N*-dimethyl aniline

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401 Fig. 4. Results of the frontal analyses.

402 Analysis Conditions: column size, 30 mm × 4.6 mm i.d.; flow rate, 2.0 mL/min; detection, UV 254 nm
403 or 293 nm; temperature, 40 °C; solid line, 4,4'-bis-(tributylammonium-methyl chloride) biphenyl
404 (BTAB) in water (500 mg/L); dotted line, benzyl tributylammonium chloride (BTBA) in water (500
405 mg/L)

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407 Fig. 5. Chromatograms of each fraction by the SPE evaluation for sulpiride solution.

408 LC Conditions: mobile phase, 100 mM formate buffer (pH 3.0) / MeCN = 2/8 (v/v); flow rate, 0.2
409 mL/min; column, TSKgel Amide-80 (150 mm × 2.0 mm i.d.); temperature, 40 °C; injection, 2.0 µL.

410 MS Conditions: nebulizer gas, N₂, 1.5 L/min; voltage of interface, 4.5 kV; voltage of detector, 1.5 kV;
411 ionization method, ESI; detection: $m/z = +342.05$.

412 SPE protocols: conditioning, water-10 mL; concentration, 10 µg/L sulpiride sol.-10 mL, washing,
413 water-1.0 mL and MeCN-1.0 mL; extraction: 100 mM formate buffer (pH 3.0) / MeCN = 2/8 (v/v)-1.0
414 mL. (a) non-treated sample (10 ppb sulpiride), (b) MeCN fraction, (c) collected fraction.

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416 Fig. 6. Flow diagram of online SPE–LC–MS with the I-MIP.

417

418 Fig. 7. Chromatogram of standard sulpiride by SPE–LC–MS

419 LC–MS conditions are the same as Fig. 5 except mobile phase, 100 mM formate buffer (pH 3.0) /
420 MeCN = 1/9 (v/v)

421 Pretreatment: conditioning with water, 4.0 mL; 10 ng/L sulphiride aqueous solution, 10 mL; washing
422 with water, 1.0 mL; washing with MeCN, 1.0 mL.

423 *Valve switching was acted after washing with MeCN and the acquirement of chromatogram was
424 started.

425

426 Fig. 8. Chromatogram of the river water sample by SPE–LC–MS

427 SPE–LC–MS conditions were the same as Fig. 7 except sample.

428 The river water was diluted to 10 times with water

Figure(s)

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

Fig. 5.

Fig. 6.

Fig. 7.

Fig. 8.

Graphical Abstract

Effective determination of a pharmaceutical, sulpiride, in river water by online SPE-LC-MS using a molecularly imprinted polymer as a preconcentration medium

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