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Uniform-sized molecularly imprinted polymer for (*S*)-ibuprofen Retention properties in aqueous mobile phases

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Abstract

A uniform-sized molecularly imprinted polymer for (*S*)-ibuprofen has been prepared by a multi-step swelling and thermal polymerization method using 4-vinylpyridine (4-VPY) and ethylene glycol dimethacrylate (EDMA) as a host functional monomer and cross-linker, respectively. The obtained (*S*)-ibuprofen imprinted 4-VPY–EDMA materials were evaluated using aqueous eluents by HPLC. Hydrophobic and hydrogen bonding interactions between ibuprofen enantiomers and 4-VPY–EDMA materials could play an important role in the retentivity and enantioselectivity. Further, partial resolution of the enantiomers of ibuprofen metabolites, 2-hydroxy- and 2-carboxyibuprofen, was attained with the (*S*)-ibuprofen imprinted 4-VPY–EDMA materials.

Keywords: Molecular imprinting; Chiral stationary phases, LC; Ibuprofen; 2-Hydroxyibuprofen; 2-Carboxyibuprofen

1. Introduction

In the molecular imprinting technique, a functional monomer(s), which allows interactions with the functional group(s) of a molecule to be recognized, is polymerized with a cross-linker(s) in the presence of the target molecule. Removal of the imprint molecule from the resulting polymer gave the molecularly imprinted complementary binding site(s) for the target molecule. The obtained molecularly imprinted polymer can be utilized for specific recognition of the target molecule as a chromatographic medium, sensor and artificial antibody [1–3]. Usually, molecularly imprinted polymers were prepared by bulk polymerization method [4]. The disadvantage of the method is that the obtained block polymers should be crushed, ground and sieved to produce packing materials. A suspension polymerization technique, based on the use of a liquid perfluorocarbon as the dispersing phase, was developed for the preparation of Boc-L-Phe (Boc=*tert*.-butyloxycarbonyl) imprinted polymers [5]. The method produced spherical polymer materials, which can be used after a simple washing, with a quantitative yield. The obtained molecularly imprinted polymer materials had molecular recognition ability similar to those prepared by the conventional bulk polymerization method. On the other hand, it was thought that water weakened the interaction between an imprint molecule and a functional monomer(s) with the suspension polymerization method using an aqueous suspension medium [6]. Recently, we prepared uniform-sized

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molecularly imprinted polymers for (*S*)-naproxen [7,8] and propranolol [9], where a typical multi-step swelling and polymerization method [10] with water as the suspension medium was used, and evaluated the obtained molecularly imprinted polymers by using a mixture of phosphate buffer and acetonitrile as an eluent. However, the molecularly imprinted polymer for (*S*)-naproxen prepared by us gave similar enantioselectivity for naproxen to that prepared with non-aqueous bulk polymerization techniques by Kempe and Mosbach [11].

In this study, we prepared uniform-sized, (*S*)-ibuprofen imprinted polymer materials using 4-vinylpyridine (4-VPY) and ethylene glycol dimethacrylate (EDMA) as the functional monomer and cross-linker, respectively, by a multi-step swelling and thermal polymerization method with water as the suspension medium. In addition, we evaluated the molecular recognition ability of the obtained (*S*)-ibuprofen imprinted 4-VPY–EDMA materials for ibuprofen, its metabolites and other 2-arylpropionic acid derivatives by using aqueous eluents. Further, the retention mechanism of ibuprofen on the 4-VPY–EDMA materials was discussed.

2. Experimental

2.1. Materials

Styrene (STY), 4-VPY and EDMA were purchased from Nacalai Tesque (Kyoto, Japan), Wako (Osaka, Japan) and Tokyo Kasei (Tokyo, Japan), respectively. Those monomers were purified by general distillation techniques in vacuo to remove the polymerization inhibitor. 2,2′-Azobis(2,4-dimethylvaleronitrile) (V-65) was purchased from Wako and used without further purification. (*S*)-(+)-Ibuprofen was purchased from Aldrich (Milwaukee, WI, USA). Racemic flurbiprofen and ibuprofen, and (*S*)-(+)-flurbiprofen were donated from Kaken (Tokyo, Japan). Racemic ketoprofen and pranoprofen were donated by Chugai (Tokyo, Japan) and Yoshitomi (Osaka, Japan), respectively. Ibuprofen metabolites, 2-[4-(2-hydroxy-2-methylpropyl)phenyl]propionic acid (2-hydroxyibuprofen) and 2-[4-(2-carboxypropyl)phenyl]propionic acid (2-carboxyibuprofen), were kindly donated by Dr. G. Geisslinger (University of Erlangen-Nürnberg, Germany). The structures of 2-arylpropionic acid derivatives and ibuprofen metabolites used in this study are illustrated in Fig. 1. Other reagents and solvents were used without further purification.

Water purified with a Nanopure II unit (Barnstead, Boston, MA, USA) was used for the preparation of the eluent and the sample solution.

2.2. Multi-step swelling and polymerization method

Uniform-sized, non-imprinted and (*S*)-ibuprofen imprinted polymer materials were prepared according to the method reported previously [7,8]. Twenty-five mmol EDMA for EDMA materials, 10 mmol STY and 25 mmol EDMA for STY–EDMA materials or 6 mmol 4-VPY and 25 mmol EDMA for

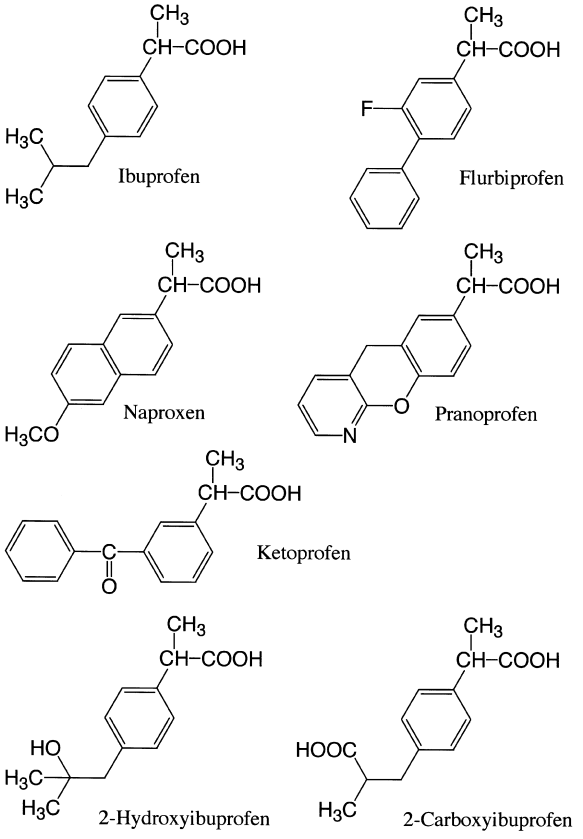

Fig. 1. Structures of 2-arylpropionic acid derivatives and ibuprofen metabolites used in this study.

4-VPY–EDMA materials were used as the functional monomer and cross-linker. When the template molecule was added, 2 mmol (*S*)-ibuprofen was admixed with the monomers.

The prepared materials were packed into a stainless steel column (100 mm×4.6 mm I.D. or 250 mm×4.6 mm I.D.) by a slurry technique using methanol as the slurry and packing solvents to evaluate their chromatographic characteristics.

2.3. Chromatography

The high-performance liquid chromatography (HPLC) system used was composed of a PU-980 pump, a UV-970 spectrophotometer (both from Jasco, Tokyo, Japan), a Rheodyne 7125 injector with a 20-μl loop (Rheodyne, Cotati, CA, USA) and a C-R6A integrator (Shimadzu, Kyoto, Japan). The flow-rate was maintained at 1.0 ml/min. Detection was performed at 200 nm. The retention factor was calculated from the equation $k=(t_R-t_M)/t_M$, where t_R and t_M are retention times of retained and unretained solutes, respectively. The retention time of unretained solute, t_M, was measured by injecting acetone. The enantioseparation factor is calculated from the equation $\alpha=k_2/k_1$, where k_1 and k_2 are the retention factors of the first and second eluted enantiomers, respectively. Resolution is calculated from the equation $R_s=2(t_{R2}-t_{R1})/(w_1+w_2)$, where t_{R1} and t_{R2} are the retention times of the first and second eluted enantiomers, respectively and w_1 and w_2 are the baseline peak widths of the first and second eluted enantiomers, respectively. Selectivity factor was calculated from the equation $S=k_{imprinted}/k_{non\text{-}imprinted}$, where $k_{imprinted}$ and $k_{non\text{-}imprinted}$ are the retention factors of a solute on the molecularly imprinted and non-imprinted polymer materials, respectively. The number of theoretical plates (N) was calculated by the equation $N=16(t_R/w)^2$. Separations were carried out at 25°C using a water bath (Thermo Minder Lt-100, Taitec, Saitama, Japan) or at 30, 50 and 70°C using a column oven (TU-310, Jasco). The eluents are prepared by using phosphoric acid, sodium dihydrogenphosphate, disodium hydrogenphosphate, trisodium phosphate and acetonitrile. The eluent used was specified in the legends of tables and figures.

3. Results and discussion

3.1. Retention properties of acidic and neutral compounds on the various EDMA materials

Fig. 2A–D, shows the effects of eluent pH on the retention properties of (*S*)-ibuprofen, benzoic acid, benzenesulfonic acid and benzene on non-imprinted EDMA, STY–EDMA and 4-VPY–EDMA, and (*S*)-ibuprofen imprinted 4-VPY–EDMA materials, respectively. The eluents used were a 3:2 mixture of 20 m*M* phosphoric acid and/or sodium phosphate, and acetonitrile. Since the first two polymers had no ionizable groups in the polymer backbone, solutes were mainly retained with hydrophobic interactions. Thus, we could estimate apparent pK_a values of benzoic acid and (*S*)-ibuprofen as reported by Horváth et al. [12]. The apparent pK_a values of benzoic acid were 5.0 and 5.1, respectively, on the EDMA and STY–EDMA materials, and those of (*S*)-ibuprofen were 5.8 and 5.7, respectively. These values were consistent with those of benzoic acid and (*S*)-ibuprofen, estimated by using an ODS column, which were 5.3 and 5.9, respectively. Benzenesulfonic acid was not retained on the EDMA and STY–EDMA materials among the eluent pH values tested. It is thought that the apparent pK_a value of benzenesulfonic acid is $<$2.3. On the other hand, benzenesulfonic acid was retained on the non-imprinted and (*S*)-ibuprofen imprinted 4-VPY–EDMA materials by decreasing the eluent pH. The retention of benzenesulfonic acid was due to ionic interactions of sulfonyl groups of benzenesulfonic acid with the positively charged 4-VPY–EDMA materials. It was reported that the apparent pK_a value of the pyridyl group of the matrix was ~4.7 [13]. However, the retention data of benzenesulfonic acid suggested the shift of the average apparent pK_a value of 4-VPY–EDMA materials to $<$3. Benzoic acid could be more retained by hydrogen bonding interaction with a pyridyl group on the non-imprinted and (*S*)-ibuprofen imprinted 4-VPY–EDMA materials than the EDMA and STY–EDMA materials. The retention factor of (*S*)-ibuprofen increased with the order of the EDMA, 4-VPY–EDMA, STY–EDMA and (*S*)-ibuprofen imprinted 4-VPY–EDMA materials. (*S*)-Ibuprofen could be mainly retained with hydrophobic interactions with EDMA and STY–EDMA materials,

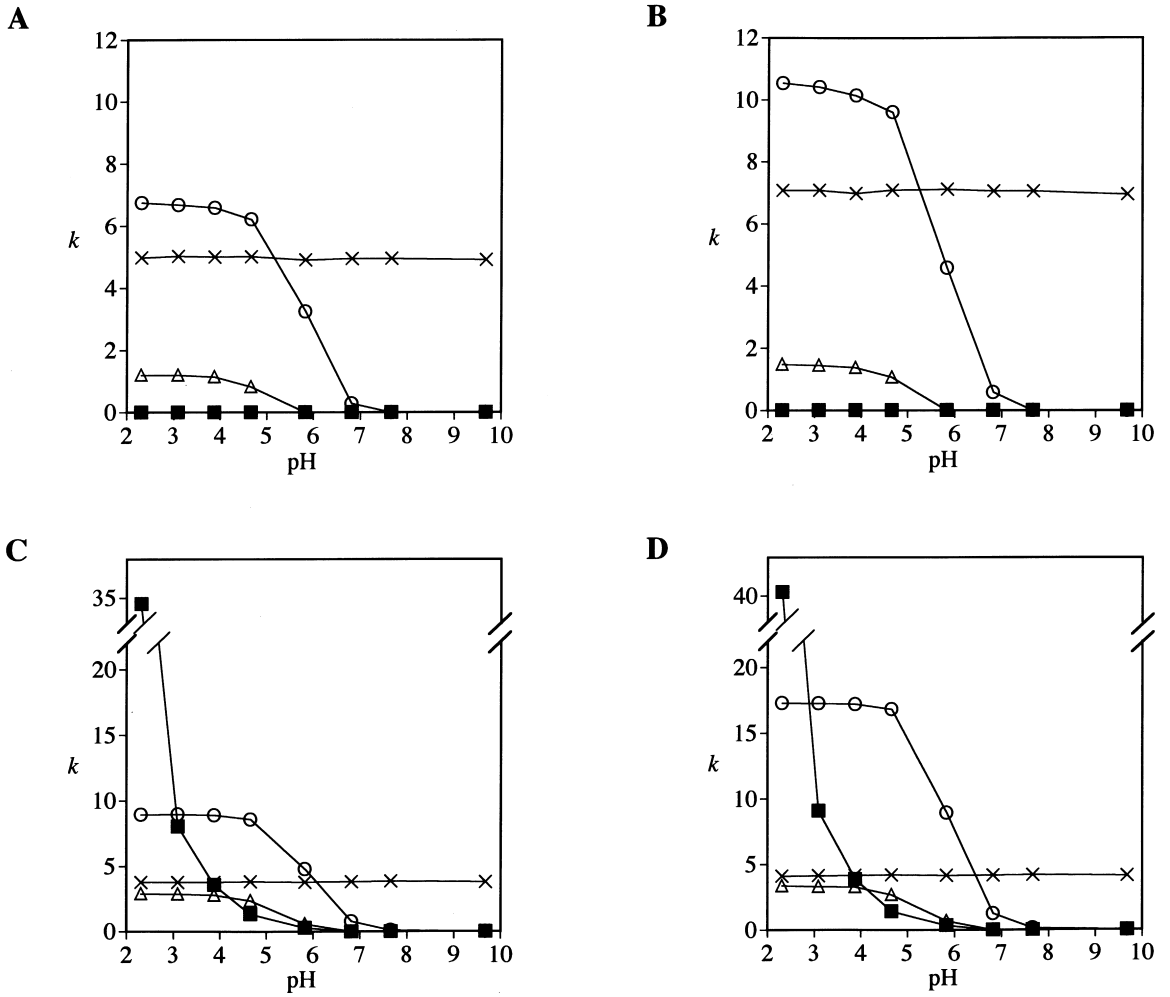

Fig. 2. Effect of eluent pH on the retention properties of (*S*)-ibuprofen, benzoic acid, benzenesulfonic acid and benzene on non-imprinted EDMA (A), STY–EDMA (B) and 4-VPY–EDMA (C), and (*S*)-ibuprofen imprinted 4-VPY–EDMA (D) materials. Key: ○–○ (*S*)-ibuprofen; △–△ benzoic acid; ■–■ benzenesulfonic acid; ×–×, benzene. HPLC conditions: column size, 100 mm×4.6 mm I.D.; column temperature, 25°C; eluent, 20 m*M* phosphoric acid and/or sodium phosphate–acetonitrile (60:40, v/v); detection, 200 nm; flow-rate, 1.0 ml/min. Loaded amount, 250 ng.

and hydrophobic and hydrogen bonding interactions with 4-VPY–EDMA materials. Further, (*S*)-ibuprofen was more retained by molecular imprinting effect on the (*S*)-ibuprofen imprinted 4-VPY–EDMA materials than the non-imprinted ones.

With regard to the retention of benzene on the EDMA, STY–EDMA and 4-VPY–EDMA materials, the retention factor increased with the order of the 4-VPY–EDMA, EDMA and STY–EDMA materials, but the retention was not affected by eluent pH values tested on those materials. These results revealed that the retention of benzene increased with an increase in hydrophobicity of polymer materials, and that the protonation of a pyridyl group did not affect the retention of benzene. The latter is due to that the extent of a pyridyl group is low in the

Table 1
Effect of eluent pH on the separation of ibuprofen enantiomers on the (*S*)-ibuprofen imprinted and non-imprinted 4-VPY–EDMA materials[a]

Eluent pH	(*S*)-Ibuprofen imprinted			Non-imprinted		
	k_2	α	R_s	k	α	R_s
2.4	7.53	1.21	0.45	3.75	1.00	–[b]
4.0	7.97	1.22	0.58	3.83	1.00	–
4.8	7.53	1.20	0.46	3.69	1.00	–
6.0	4.15	1.14	<0.3	2.15	1.00	–
7.0	1.01	1.00	–	0.56	1.00	–
7.8	0.14	1.00	–	0.13	1.00	–

[a] HPLC conditions: column size, 100 mm×4.6 mm I.D.; column temperature, 25°C; eluent, 20 m*M* phosphoric acid and/or sodium phosphate–acetonitrile (50:50, v/v); flow-rate, 1.0 ml/min. Loaded amount, 100 ng.
[b] Not enantioseparated.

4-VPY–EDMA materials, and/or that the extent of the protonation of a pyridyl group is not so large in the eluent pH values tested.

3.2. Separation of ibuprofen enantiomers on the (S)-ibuprofen imprinted 4-VPY–EDMA materials

Table 1 shows the effect of eluent pH on the separation of ibuprofen enantiomers on the (*S*)-ibuprofen imprinted and non-imprinted 4-VPY–EDMA materials. The non-imprinted 4-VPY–EDMA materials had no chiral recognition ability toward ibuprofen, while the (*S*)-ibuprofen imprinted 4-VPY–EDMA materials showed enantioselectivity for ibuprofen. The constant retentivity and enantioselectivity were obtained with eluent pH between 2.3 and 4.6. As described above, in this pH range ibuprofen enantiomers were uncharged and 4-VPY–EDMA

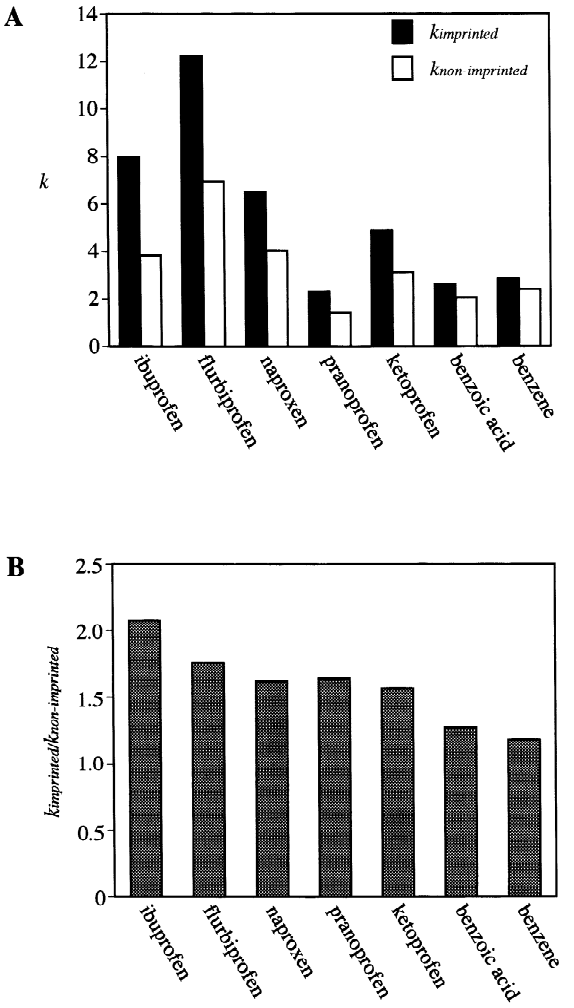

Fig. 3. Selectivity of the (*S*)-ibuprofen imprinted 4-VPY–EDMA materials toward 2-arylpropionic acid derivatives, and acidic and neutral compounds. HPLC conditions as in Table 1. Loaded amount, 100 ng.

Table 2
Effect of acetonitrile content on the separation of ibuprofen enantiomers on the (*S*)-ibuprofen imprinted and non-imprinted 4-VPY–EDMA materials[a]

Acetonitrile content (%)	(*S*)-Ibuprofen imprinted			Non-imprinted		
	k_2	α	R_s	k	α	R_s
40	23.18	1.26	0.90	11.29	1.00	–[b]
50	7.97	1.22	0.58	3.83	1.00	–
60	3.19	1.18	0.41	1.60	1.00	–
70	1.43	1.00	–	0.82	1.00	–

[a] HPLC conditions as in Table 1 except the eluent used is a mixture of 20 m*M* phosphoric acid–sodium dihydrogen phosphate (pH 3.2) and acetonitrile.
[b] Not enantioseparated.

materials were partially protonated. These results reveal that there are almost no differences in the interactions of uncharged ibuprofen enantiomers with 4-VPY–EDMA materials despite proceeding of the protonation of a pyridyl group with a decrease in the eluent pH. However, it is plausible that with a further decrease in the eluent pH the retentivity and enantioselectivity could be decreased. Table 2 shows the effect of acetonitrile content on the separation of ibuprofen enantiomers on the (*S*)-ibuprofen imprinted and non-imprinted 4-VPY–EDMA materials. With an increase in the acetonitrile content, the retentivity and enantioselectivity of ibuprofen enantiomers decreased.

The results obtained above suggest that hydrophobic and hydrogen bonding interactions between ibuprofen enantiomers and 4-VPY–EDMA materials could play an important role in the retentivity and enantioselectivity.

3.3. Selectivity of the (S)-ibuprofen imprinted 4-VPY–EDMA materials

Selectivities of the (*S*)-ibuprofen imprinted 4-VPY–EDMA materials toward 2-arylpropionic acid derivatives, and acidic and neutral compounds were examined. Fig. 3A and B shows the retention factors toward 2-arylpropionic acid derivatives, and acidic and neutral compounds on the (*S*)-ibuprofen imprinted and non-imprinted 4-VPY–EDMA materials, and selectivity factor ($k_{\text{imprinted}}/k_{\text{non-imprinted}}$), respectively, where the eluent used is 20 m*M* phosphoric acid–sodium dihydrogenphosphate (pH 3.2)–acetonitrile (50:50, v/v). Selectivity factor for (*S*)-ibuprofen is 2.08, and those for other 2-arylpropionic acid derivatives, flurbiprofen, naproxen, pranoprofen and ketoprofen, are 1.76, 1.62, 1.64 and 1.57, respectively. The (*S*)-ibuprofen imprinted 4-VPY–EDMA materials gave the highest selectivity for (*S*)-ibuprofen. Separation of ibuprofen enantiomers was attained, as described above. Further, flurbiprofen, which gave higher selectivity factor than naproxen, pranoprofen and ketoprofen, showed partial resolution of those enantiomers with enantioseparation factor of 1.11. The elution order of flurbiprofen enantiomers was (*R*)/(*S*). On the other hand, no chiral resolution of naproxen, pranoprofen and ketoprofen, which gave lower selectivity factor, was observed. Selectivity factor for an acidic compound, benzoic acid, was 1.27. Selectivity factor for a neutral compound, benzene was 1.18. With regard to selectivities for basic compounds, those were not retained on the non-imprinted and (*S*)-ibuprofen imprinted materials at eluent pH of 4.0.

3.4. Separation of ibuprofen enantiomers and other 2-arylpropionic acid derivatives on the (S)-ibuprofen imprinted 4-VPY–EDMA materials

Fig. 4A and B shows the separation of pranoprofen, ketoprofen, and (*R*)- and (*S*)-ibuprofen on the non-imprinted and (*S*)-ibuprofen imprinted 4-VPY–EDMA materials, respectively. Separation of racemic ketoprofen and ibuprofen was incomplete on the non-imprinted materials, while on the (*S*)-ibuprofen imprinted materials, racemic ketoprofen and ibuprofen enantiomers were separated one another.

Fig. 5A–C shows the separation of ibuprofen enantiomers on the (*S*)-ibuprofen imprinted 4-VPY–EDMA materials at column temperatures of 30, 50 and 70°C, respectively. Table 3 shows the retention factor, enantioseparation factor, resolution and the number of theoretical plates of ibuprofen enantiomers shown in Fig. 5. With an increase in the column temperature, the retention factor and enantioseparation factor were decreased, while the highest resolution was obtained at a column temperature of 50°C. This is due to the suppression of the band-broadening of the second-eluted enantiomer, (*S*)-ibuprofen. The column performance of the (*S*)-ibuprofen imprinted 4-VPY–EDMA materials was improved by elevating a column temperature, as reported by Sellergren [14].

3.5. Enantioseparation of 2-hydroxy- and 2-carboxyibuprofen on the (S)-ibuprofen imprinted 4-VPY–EDMA materials

The obtained (*S*)-ibuprofen imprinted 4-VPY–EDMA materials were applied to chiral resolution of two major metabolites of ibuprofen, 2-hydroxy- and 2-carboxyibuprofen. Table 4 shows the retention factor, enantioseparation factor, resolution and selectivity factor of ibuprofen and its metabolites on the (*S*)-ibuprofen imprinted and non-imprinted 4-VPY–EDMA materials. Partial resolution of 2-hydroxy-

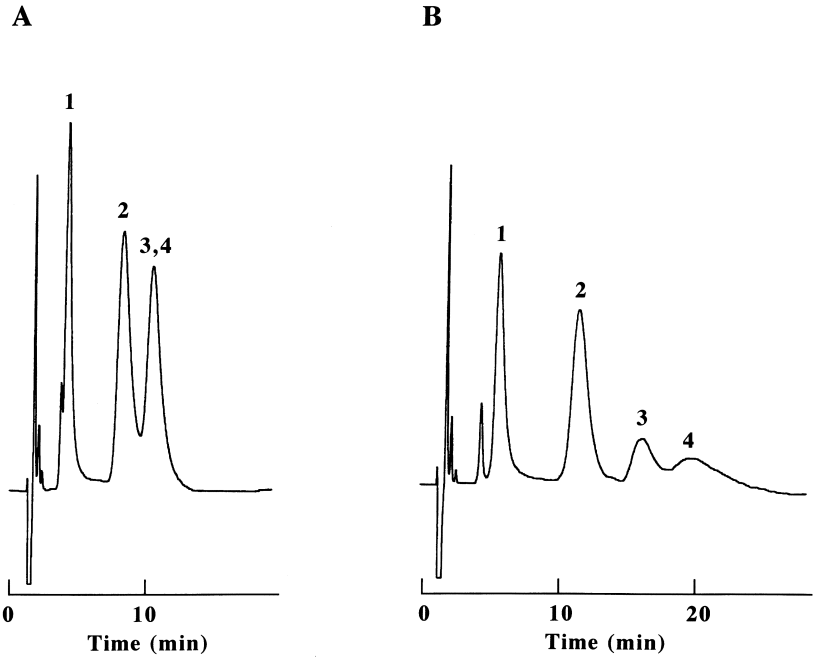

Fig. 4. Separation of pranoprofen, ketoprofen and ibuprofen enantiomers on the non-imprinted (A) and (*S*)-ibuprofen imprinted (B) 4-VPY–EDMA materials. Key: 1=racemic pranoprofen; 2=racemic ketoprofen; 3=(*R*)-ibuprofen; 4=(*S*)-ibuprofen. HPLC conditions as in Fig. 2 except the eluent used is 20 m*M* phosphoric acid–sodium dihydrogenphosphate (pH 3.2)–acetonitrile (55:45, v/v). Loaded amounts: racemic pranoprofen, 20 ng; racemic ketoprofen and ibuprofen, 40 ng.

ibuprofen enantiomers was attained despite less retentions of these metabolites than ibuprofen. Although 2-carboxyibuprofen had four stereoisomers because of the introduction of the second chiral center in the molecule, only two peaks were observed on the (*S*)-ibuprofen imprinted 4-VPY–EDMA materials.

4. Conclusion

A uniform-sized molecularly imprinted polymer for (*S*)-ibuprofen was prepared using 4-VPY and EDMA as a functional monomer and cross-linker, respectively. The (*S*)-ibuprofen imprinted 4-VPY–EDMA materials showed the highest selectivity for (*S*)-ibuprofen. Hydrophobic and hydrogen bonding interactions between ibuprofen enantiomers and 4-VPY–EDMA materials could play an important role in the retentivity and enantioselectivity with the use of aqueous eluents. Further, partial resolution of the enantiomers of ibuprofen metabolites, 2-hydroxy- and 2-carboxyibuprofen, was attained with the (*S*)-ibuprofen imprinted 4-VPY–EDMA materials.

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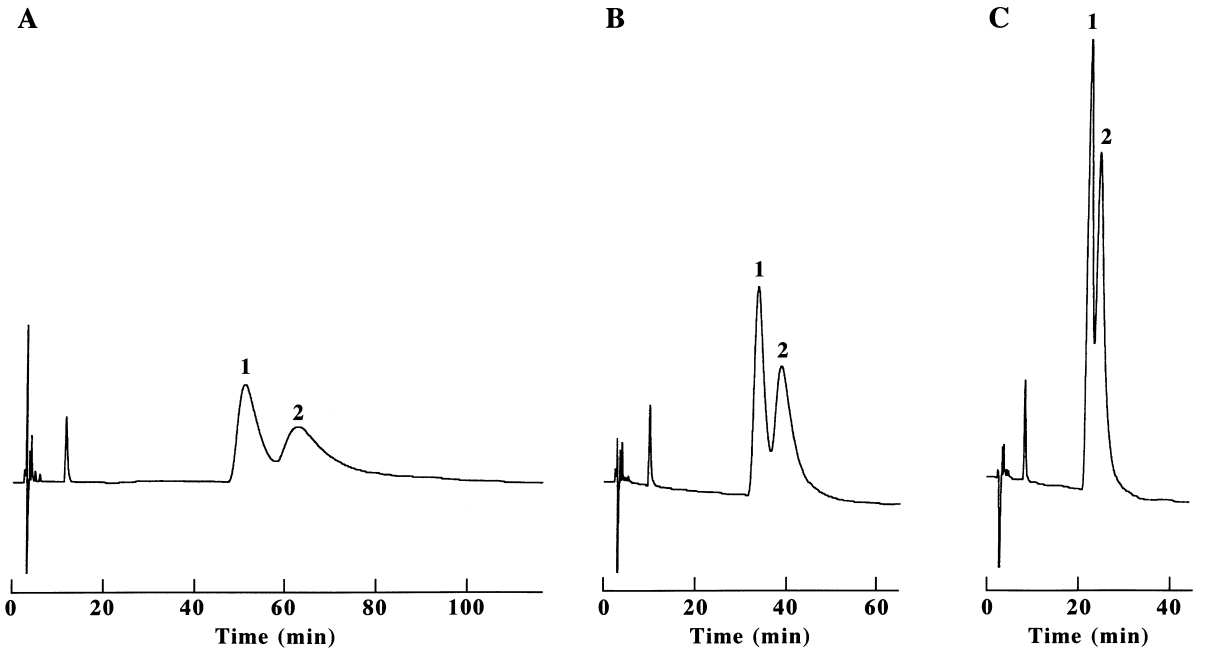

Fig. 5. Separation of ibuprofen enantiomers on the (*S*)-ibuprofen imprinted 4-VPY–EDMA materials at column temperatures of 30 (A), 50 (B) and 70°C (C). Key: 1=(*R*)-ibuprofen; 2=(*S*)-ibuprofen. HPLC conditions as in Fig. 2 except the column size is 250 mm×4.6 mm I.D., and the eluent used is 20 m*M* phosphoric acid–sodium dihydrogenphosphate (pH 3.2)–acetonitrile (60:40, v/v). Loaded amounts: racemic ibuprofen, 250 ng.

Table 3
Effect of column temperature on the separation of ibuprofen enantiomers on the (*S*)-ibuprofen imprinted 4-VPY–EDMA materials[a]

Column temperature (°C)	k_1	k_2	α	R_s	N_1	N_2
30	14.98	18.61	1.24	0.80	570	179
50	9.34	10.95	1.17	0.84	959	380
70	6.00	6.68	1.11	0.75	1718	754

[a] HPLC conditions as in Fig. 5.

Table 4
Retention factor, enantioseparation factor and selectivity factor of ibuprofen and its metabolites on the (*S*)-ibuprofen imprinted and non-imprinted 4-VPY–EDMA materials[a]

Solute	(*S*)-Ibuprofen imprinted			Non-imprinted		Selectivity factor
	k_1	k_2	α	k	α	
Ibuprofen	82.75	106.8	1.29	49.39	1.00	2.16
2-Hydroxyibuprofen	4.80	5.30	1.10	2.90	1.00	1.83
2-Carboxyibuprofen	13.51	14.81	1.10	7.92	1.00	1.87

[a] HPLC conditions as in Table 1 except the eluent used is 20 m*M* phosphoric acid–sodium dihydrogenphosphate (pH 3.2)–acetonitrile (70:30, v/v). Loaded amount, 100 ng.

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