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Journal of Chromatography A, 948 (2002) 77–84

JOURNAL OF CHROMATOGRAPHY A

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Uniformly sized molecularly imprinted polymer for *d*-chlorpheniramine Evaluation of retention and molecular recognition properties in an aqueous mobile phase

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Abstract

A uniformly sized molecularly imprinted polymer (MIP) for *d*-chlorpheniramine has been prepared by a multi-step swelling and polymerization method using methacrylic acid and ethylene glycol dimethacrylate as a functional monomer and cross-linker, respectively. The retentive and enantioselective properties of chlorpheniramine and its structurally related compounds on the MIP were evaluated using an aqueous mobile phase. Electrostatic and hydrophobic interactions could mainly work for the retention and enantioseparation of chlorpheniramine in aqueous mobile phase. Further, the MIP showed the highest recognition for chlorpheniramine and slight recognition for its structurally related compounds, and enantioseparation of pheniramine was attained.

Keywords: Molecular imprinting; Stationary phases, LC; Enantiomer separation; Chlorpheniramine

1. Introduction

Since the molecular imprinting techniques can afford the complementary binding site(s) for a template molecule, the molecularly imprinted polymers (MIPs) are used for chromatographic separations, solid-phase extractions, membranes, antibody-mimics and sensors for the purpose of specific recognition of the target molecule [1–3]. Usually, non-aqueous bulk polymerization methods [4] have been utilized to obtain MIPs. The disadvantage of the method is that the obtained block polymers had to be crushed, ground and sieved to produce packing materials. The MIPs obtained are unsuitable as high-performance liquid chromatography (HPLC) packing materials owing to random shape and size distribution. Recently, we prepared uniformly sized MIPs for (*S*)-naproxen [5], -ibuprofen [6] and -propranolol [7] using a multi-step swelling and polymerization method. The advantages of the method are easy to prepare uniformly sized and monodispersed particles, suitable as HPLC packing materials, and easy to perform in situ modification. Our MIP for (*S*)-naproxen [5,8] gave similar enantioselectivity for naproxen to that prepared with non-aqueous bulk polymerization techniques by Kempe and Mosbach [9]. Further, our MIP gave molecular recognition not only for the template molecule but also for its structurally related compounds such as drug metabolites [6,7].

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0021-9673/02/$ – see front matter
PII: S0021-9673(01)01262-6

Chlorpheniramine is an antihistaminic H_1 drug. *d*-Chlorpheniramine has been shown to be 200-fold more active than its enantiomer in vivo in protecting guinea-pig against histamine [10]. A few reports have shown interspecies differences in the stereoselective pharmacokinetics of chlorpheniramine [11,12]. There is no report for the preparation of an MIP for chlorpheniramine. In this study, we prepared the MIP for *d*-chlorpheniramine using methacrylic acid (MAA) and ethylene glycol dimethacrylate (EDMA) as a functional monomer and cross-linker, respectively, and evaluated the retentive and enantioselective properties for chlorpheniramine and its structurally related compounds on the MIP using aqueous mobile phase. Further, the retention and enantioseparation mechanism of chlorpheniramine on the MIP was discussed.

2. Experimental

2.1. Materials

EDMA and MAA were purchased from Tokyo Chemical Industry (Tokyo, Japan) and Wako (Osaka, Japan), respectively. These monomers were purified by general distillation techniques in vacuo to remove the polymerization inhibitor. 2,2′-Azobis(2,4-dimethylvaleronitrile) was purchased from Wako (Tokyo, Japan). *d*-Chlorpheniramine, propranolol and phenyltrimethylammonium chloride were purchased from Sigma–Aldrich Japan (Tokyo, Japan). Pheniramine was purchased from Nacalai Tesque (Kyoto, Japan). Homochlorcyclizne was donated by Eisai (Tokyo, Japan). The structures of chlorpheniramine and structurally related compounds used in this study are illustrated in Fig. 1. A Capcell Pak C_{18} column (15 cm×4.6 mm I.D.) was kindly donated by Shiseido (Tokyo, Japan). 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.

Table 1
Molar amounts of template, functional monomer and cross-linker used for the preparation of MIPs for *d*-chlorpheniramine

MIP	Amount (mmol)		
	Template	MAA	EDMA
1	2	3.5	25
2	2	7	25
3	2	14	25
4	1	3.5	25
5	4	14	25

2.2. Multi-step swelling and polymerization method

Preparation of the uniformly sized, macroporous MIP for *d*-chlorpheniramine as well as the non-imprinted polymer by a multi-step swelling and polymerization method was carried out as reported previously [13,14]. The molar amounts of *d*-chlorpheniramine, MAA and EDMA as shown in Table 1 were used as a template, functional monomer and

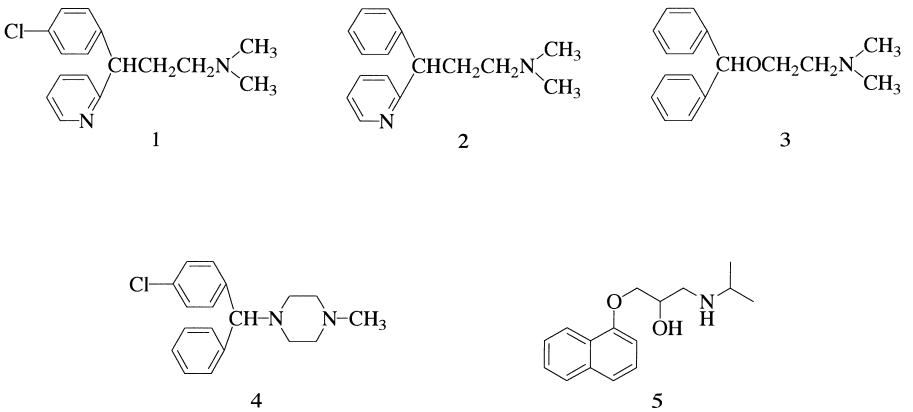

Fig. 1. Structures of chlorpheniramine and its structurally related compounds used in this study. 1, Chlorpheniramine; 2, pheniramine; 3, diphenhydramine; 4, homochlorcyclizine; 5, propranolol.

cross-linker, respectively. The prepared polymers were packed into a stainless steel column (100 mm × 4.6 mm I.D.) by a slurry packing technique using methanol as the slurry and packing solvents to evaluate their chromatographic characteristics.

2.3. Chromatography

The 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 0.5, 0.8, 1.0 or 2.0 ml/min. Detection was performed at 200 nm. The retention factor was calculated from the equation $k=(t_r-t_0)/t_0$, where t_r and t_0 are retention times of retained and unretained solutes, respectively. The retention time of unretained solute, t_0, was measured by injecting acetone or tyrosine. The enantioseparation factor is calculated from the equation $\alpha=k_d/k_R$, where k_R and k_d are the retention factors of the first and second eluted enantiomers, respectively. Resolution is calculated from the equation $R_s=2(t_d-t_R)/(w_R+w_d)$, where t_R and t_d are the retention times of the first and second eluted enantiomers, respectively, and w_R and w_d are the baseline peak widths of the first and second eluted enantiomers, respectively. The number of theoretical plates (N) was calculated by the equation $N=16(t_r/w)^2$. The selectivity factor was calculated from the equation $S=k_{\text{imprinted}}/k_{\text{non-imprinted}}$, where $k_{\text{imprinted}}$ and $k_{\text{non-imprinted}}$ are the retention factors of a solute on the MIP and non-imprinted polymer, respectively. Separations were carried out at 25, 30, 40, 50, 60 or 70°C using a column oven (TU-310, Jasco). The eluents are prepared by using phosphoric acid, potassium dihydrogenphosphate, dipotassium hydrogenphosphate and acetonitrile. The eluent used was specified in the legends of tables and figures.

3. Results and discussion

3.1. Effect of molar amounts of d-chlorpheniramine and MAA on chiral resolution of chlorpheniramine

In this study, we tried to optimize the preparation of the MIP for *d*-chlorpheniramine and the separation of chlorpheniramine enantiomers on the MIP. Table 2 shows the effect of molar amounts of *d*-chlorpheniramine and MAA on the retention factor, enantioseparation factor and resolution of chlorpheniramine, where EDMA used is 25 mmol. The eluent used was 50 m*M* potassium dihydrogenphosphate + dipotassium hydrogenphosphate (pH 5.1)–acetonitrile (30:70, v/v). When the template molecule, *d*-chlorpheniramine, used was 2 mmol, the retention factor of *d*-chlorpheniramine increased with an increase in the functional monomer amount used (see MIPs 1, 2 and 3). However, the maximum enantioselectivity was observed with the use of 7 mmol of MAA. Excess functional monomer resulted in the non-specific interaction and inferior chiral recognition of chlorpheniramine. When the molar ratio of *d*-chlorpheniramine and MAA is 3.5, the retentivity and enantioselectivity of chlorpheniramine increased with an increase in their amounts (see MIPs 2, 4, and 5). When *d*-chlorpheniramine and MAA used were 2 and 7 mmol, respectively, the resolution of chlorpheniramine was better despite the shorter retention times. In the following study, the amounts of *d*-chlorpheniramine, MAA and EDMA used were 2, 7 and 25 mmol, respectively.

Table 2
Effect of molar amounts of *d*-chlorpheniramine and MAA on retention factor, enantioseparation factor and resolution of chlorpheniramine

MIP	k_R	k_d	α	R_s
1	3.06	4.00	1.30	0.33
2	10.9	20.4	1.88	0.54
3	22.0	33.4	1.52	–
4	2.58	3.48	1.35	0.35
5	29.1	59.9	2.06	0.55

HPLC conditions: column size, 100 mm × 4.6 mm I.D.; column temperature, 25°C; eluent, 50 m*M* potassium dihydrogenphosphate + dipotassium hydrogenphosphate (pH 5.1)–acetonitrile (30:70, v/v); flow-rate, 1.0 ml/min; loaded amount, 1000 ng.

3.2. Retention properties of various compounds on the MAA–EDMA polymers

Fig. 2A–C shows the effects of eluent pH on the retention properties of benzene, phenol and benzoic acid on *d*-chlorpheniramine-imprinted MAA–EDMA, non-imprinted MAA–EDMA and ODS columns, respectively. The eluent used was 50 m*M* phosphoric acid and/or potassium phosphate–ace-

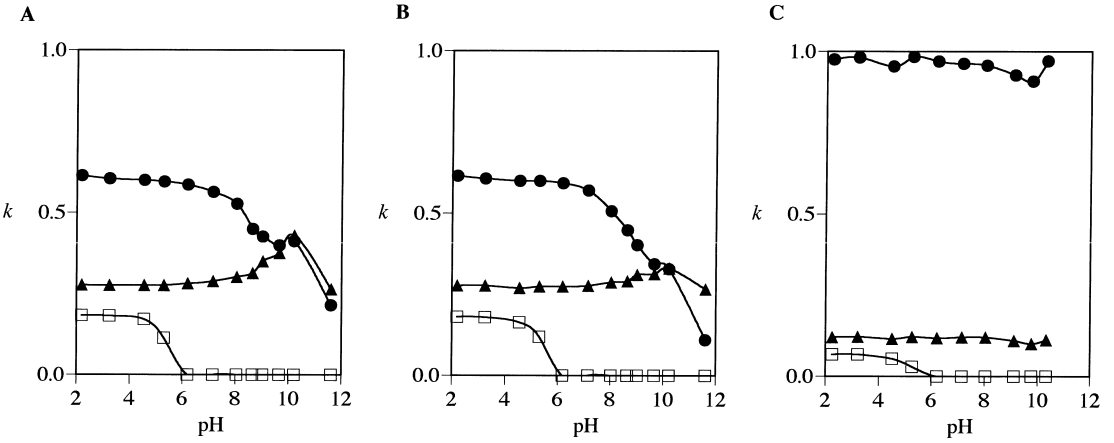

Fig. 2. Effect of eluent pH on the retention properties of benzene, phenol and benzoic acid on *d*-chlorpheniramine-imprinted MAA–EDMA (A), non-imprinted MAA–EDMA (B) and ODS (C) columns. Key: ●–●, benzene; ▲–▲, phenol; □–□, benzoic acid. HPLC conditions: column size, 100 mm×4.6 mm I.D.; column temperature, 25°C; eluent, 50 m*M* phosphoric acid+potassium dihydrogenphosphate or potassium dihydrogenphosphate+dipotassium hydrogenphosphate–acetonitrile (30:70, v/v); detection, 200 nm; flow-rate, 1.0 ml/min. Loaded amount, 1000 ng.

tonitrile (30:70, v/v). On the first two MAA–EDMA columns, the retention factor of benzene was slightly decreased in the eluent pH ranges 4–8, while a further increase in the eluent pH resulted in the gradual decrease in the retention factor of benzene. This is due to dissociation of carboxylic acids in the highly cross-linked MAA–EDMA polymers, whose average pK_a value was reported to be about 9 in the eluent including 70% acetonitrile [15]. A decrease in the retention factor of phenol in the eluent pH ranges 10–12 on the MAA–EDMA polymers is due to the dissociation of the phenolic hydroxyl group. On the other hand, the retention factors of benzene and phenol on the ODS column remained unchanged in the eluent pH ranges 2–10. Since the ODS groups were unionizable, the uncharged solutes, benzene and phenol, were retained with hydrophobic interactions in the eluent pH ranges tested. With regard to the retention of benzoic acid, the similar retention properties were obtained among the three columns. Previously, we reported that the apparent pK_a value of benzoic acid was ca. 5 [6]. The retention of benzoic acid could be explained by hydrophobic interactions.

Fig. 3A–C shows the effects of eluent pH on the retention properties of *d*-chlorpheniramine, propranolol and phenyltrimethylammonium chloride on *d*-chlorpheniramine-imprinted MAA–EDMA, non-imprinted MAA–EDMA and ODS columns, respectively. On the first two columns, the retention factor of phenyltrimethylammonium chloride, which is completely dissociated among the eluent pH values tested, increased with an increase in the eluent pH and gave a plateau above the eluent pH 10. With regard to the retentions of *d*-chlorpheniramine and propranolol, the maximum retentions of them were attained at eluent pH values of 8.0 and 8.5, respectively. By taking account into the pK_a values of chlorpheniramine and propranolol (9.2 and 9.5, respectively) [16] and the average pK_a value of cross-linked MAA–EDMA polymers (ca. 9), the retention properties of these basic compounds on the columns could be easily elucidated. With an increase of the eluent pH, phenyltrimethylammonium chloride, *d*-chlorpheniramine and propranolol were more retained due to the ionic interactions of amine groups of them with the negatively charged MAA–EDMA polymers. Then, the plateau of the retention of phenyltrimethylammonium chloride was observed because of complete dissociation of MAA–EDMA materials. With regard to the retentions of *d*-chlorpheniramine and propranolol, the maximum retentions of them were observed at around the pK_a values, and then the drastic decreases of the retentions were observed with further increase in the eluent pH, because of deprotonation of the amine

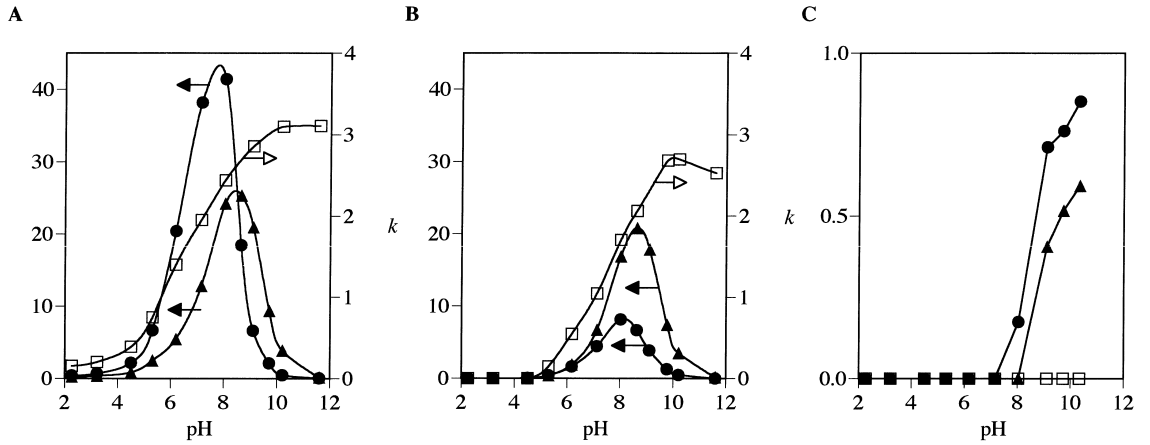

Fig. 3. Effect of eluent pH on the retention properties of *d*-chlorpheniramine, propranolol and phenyltrimethylammonium chloride on *d*-chlorpheniramine-imprinted MAA–EDMA (A), non-imprinted MAA–EDMA (B) and ODS (C) columns. Key: ●–●, *d*-chlorpheniramine; ▲–▲, propranolol; □–□, phenyltrimethylammonium chloride. HPLC conditions as in Fig. 2.

groups. Further, *d*-chlorpheniramine was more retained by molecular imprinting effect on the *d*-chlorpheniramine-imprinted MAA–EDMA columns than the non-imprinted ones. The obtained results agreed well with those reported by Sellergren and Shea [15]. On the other hand, the retentions of these basic compounds on the ODS column could be explained by hydrophobic interactions.

3.3. Separation of chlorpheniramine enantiomers on the d-chlorpheniramine-imprinted MAA–EDMA columns

Table 3 shows the effect of eluent pH on the separation of chlorpheniramine enantiomers on the *d*-chlorpheniramine-imprinted MAA–EDMA columns. The non-imprinted MAA–EDMA columns had no chiral recognition ability toward chlorpheniramine, while the *d*-chlorpheniramine-imprinted MAA–EDMA columns showed enantioselectivity for chlorpheniramine. The highest enantioselectivity and resolution were obtained with eluent pH between 6.2 and 8.0. As described above, in this pH range chlorpheniramine enantiomers were charged and MAA–EDMA polymers were partially charged. With regard to the effect of acetonitrile content on the separation of chlorpheniramine enantiomers on the *d*-chlorpheniramine-imprinted MAA–EDMA columns, the retentivity of chlorpheniramine enantiomers decreased with an increase in the acetonitrile content, while the enantioselectivity remained unchanged (data not shown). When only acetonitrile was used as the eluent, the enantioseparation of chlorpheniramine was not attained. The results obtained above suggest that ionic and hydrophobic interactions could play an important role in the retentivity and enantioselectivity of chlorpheniramine enantiomers on the MAA–EDMA columns.

3.4. Selectivity of the d-chlorpheniramine-imprinted MAA–EDMA polymers

Selectivities of the *d*-chlorpheniramine-imprinted

Table 3
Effect of eluent pH on retention factor, enantioseparation factor and resolution of chlorpheniramine

Eluent pH	k_R	k_d	α	R_s
3.2	0.68	0.68	1.00	–
4.5	1.58	2.17	1.38	0.39
5.3	4.14	6.63	1.60	0.42
6.2	10.9	20.4	1.88	0.54
7.2	20.2	38.2	1.89	0.52
8.0	22.4	41.4	1.85	0.54
8.6	18.5	18.5	1.00	–

HPLC conditions: column size, 100 mm×4.6 mm I.D.; column temperature, 25°C; flow-rate, 1.0 ml/min; detection, 200 nm; eluent, 50 m*M* phosphoric acid+potassium dihydrogenphosphate or potassium dihydrogenphosphate+dipotassium hydrogenphosphate–acetonitrile (30:70, v/v).

Table 4
Retentivity and selectivity of the *d*-chlorpheniramine-imprinted MAA–EDMA polymers toward *d*-chlorpheniramine and its structurally related compounds

	$k_{imprinted}$	$k_{non\text{-}imprinted}$	Selectivity factor
d-Chlorpheniramine	38.77	4.70	8.24
Pheniramine	16.04	3.33	4.82
Diphenhydramine	8.56	3.63	2.36
Homochlorcyclizine	18.33	7.49	2.45

HPLC conditions as in Fig. 2 except that the eluent used is potassium dihydrogenphosphate + dipotassium hydrogenphosphate (pH 6.0)–acetonitrile (30:70, v/v).

MAA–EDMA polymers toward *d*-chlorpheniramine, its structurally related compounds, and acidic and neutral compounds were examined. Table 4 shows the retention factors for *d*-chlorpheniramine and its structurally related compounds on the *d*-chlorpheniramine-imprinted and non-imprinted MAA–EDMA columns, and the selectivity factors [$k_{imprinted}/k_{non\text{-}imprinted}$], where the eluent used is 50 m*M* potassium dihydrogenphosphate + dipotassium hydrogenphosphate (pH 6.0)–acetonitrile (30:70, v/v). Selectivity factor for *d*-chlorpheniramine is 8.57, and those for its structurally related compounds pheniramine, diphenhydramine and homochlorcyclizne, are 4.82, 2.36, and 2.45, respectively. However, those of propranolol and phenyltrimethylethylammonium chloride were 1.95 and 1.87, respectively. The *d*-chlorpheniramine-imprinted MAA–EDMA polymers gave the highest selectivity for *d*-chlorpheniramine. Separation of chlorpheniramine enantiomers was attained, as described above. Further, only pheniramine showed partial resolution with an enantioseparation factor of 1.26. On the other hand, no chiral resolution of homochlorcyclizine was observed. Selectivity factors for other compounds, benzene and phenol, were 0.99 and 1.02, respectively. With regard to selectivity of an acidic compound, benzoic acid was not retained on the MAA–EDMA columns at eluent pH of 6.2.

3.5. Effect of column temperature and flow-rate on the separation of chlorpheniramine enantiomers on the d-chlorpheniramine-imprinted MAA–EDMA columns

Fig. 4A–C shows the separation of chlorpheniramine enantiomers on the MIP for *d*-chlorpheniramine at column temperatures of 30, 50 and 70°C, respectively. Table 5 shows the retention factor, enantioseparation factor, resolution and the number of theoretical plates of chlorpheniramine enantiomers at column temperatures of 30, 40, 50, 60 and 70°C. With an increase in the column temperature, the retention factor and enantioseparation factor de-

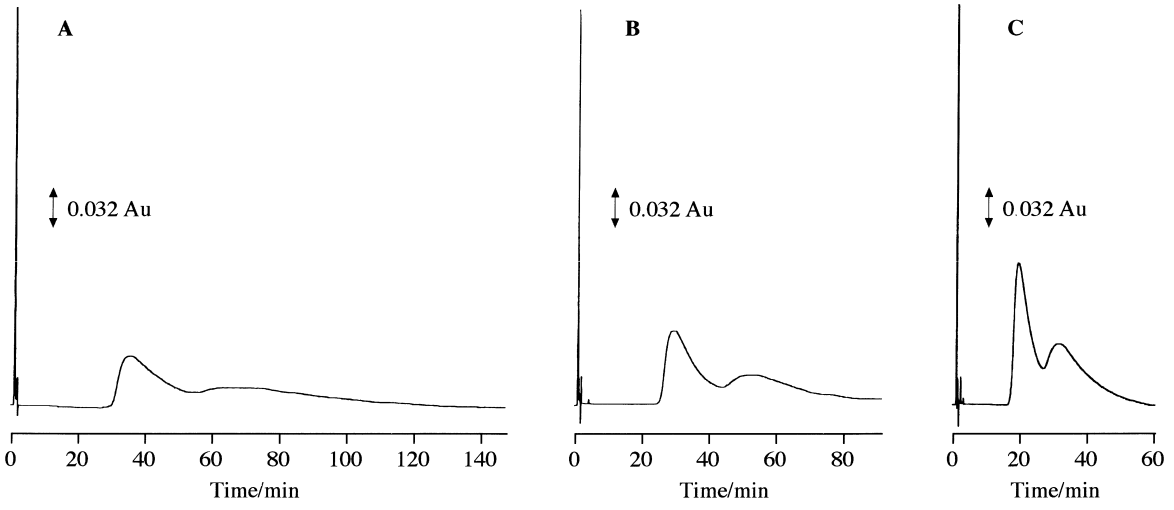

Fig. 4. Separation of chlorpheniramine enantiomers on the *d*-chlorpheniramine-imprinted MAA–EDMA columns at column temperatures of 30 (A), 50 (B) and 70°C (C). Key: 1, *R*-chlorpheniramine; 2, *d*-chlorpheniramine. HPLC conditions as in Table 5.

Table 5
Effect of column temperature on retention factor, enantioseparation factor, resolution and the number of theoretical plates of chlorpheniramine

Column temperature (°C)	k_R	k_d	α	R_s	N_R	N_d
30	25.93	48.22	1.86	0.75	43	21
40	24.41	44.68	1.83	0.76	48	22
50	22.16	39.53	1.78	0.77	57	24
60	17.66	32.29	1.83	0.76	58	22
70	14.27	23.75	1.66	0.74	73	29

HPLC conditions: column size, 100 mm×4.6 mm I.D.; flow-rate, 1.0 ml/min; detection, 200 nm; eluent, 50 m*M* potassium dihydrogenphosphate + dipotassium hydrogenphosphate (pH 6.0)–acetonitrile (30:70, v/v); loaded amount, 3000 ng.

Table 6
Effect of flow-rate on retention factor, enantioseparation factor, resolution and the number of theoretical plates of chlorpheniramine

Flow-rate (ml/min)	k_R	k_d	α	R_s	N_R	N_d
0.5	14.05	23.62	1.68	0.83	89	35
0.8	14.11	24.24	1.72	0.81	70	32
1.0	14.27	23.75	1.66	0.74	73	29
2.0	16.40	28.85	1.75	0.56	50	12

HPLC conditions: column size, 100 mm×4.6 mm I.D.; column temperature, 70°C; eluent, 50 m*M* potassium dihydrogenphosphate + dipotassium hydrogenphosphate (pH 6.0)–acetonitrile (30:70, v/v); loaded amount is 3000 ng.

creased, while the resolution remained unchanged. This is due to the suppression of the band-broadening of the second-eluted enantiomer, *d*-chlorpheniramine. Fig. 5A–C shows the separation of chlorpheniramine enantiomers on the MIP for *d*-chlorpheniramine at flow-rates of 0.5, 0.8 and 2.0 ml/min, respectively. Table 6 shows the retention factor, enantioseparation factor, resolution and the number of theoretical plates of chlorpheniramine enantiomers at flow-rates of 0.5, 0.8, 1.0 and 2.0 ml/min. With a decrease in the flow-rate, the retention times increased, while the highest resolution was obtained at a flow-rate of 0.5 ml/min. This could be due to the slow mass transfer of chlorpheniramine enantiomers on the MIP. As described above, the column performance of the MIP for *d*-chlorpheniramine was improved by elevating the column temperature and decreasing the flow-rate as reported previously [17].

4. Conclusion

A uniformly sized molecularly imprinted polymer for *d*-chlorpheniramine was prepared using MAA and EDMA as a functional monomer and cross-linker, respectively, and evaluated using a mixture of phosphate buffer and acetonitrile as an eluent. When the amounts of *d*-chlorpheniramine, MAA and

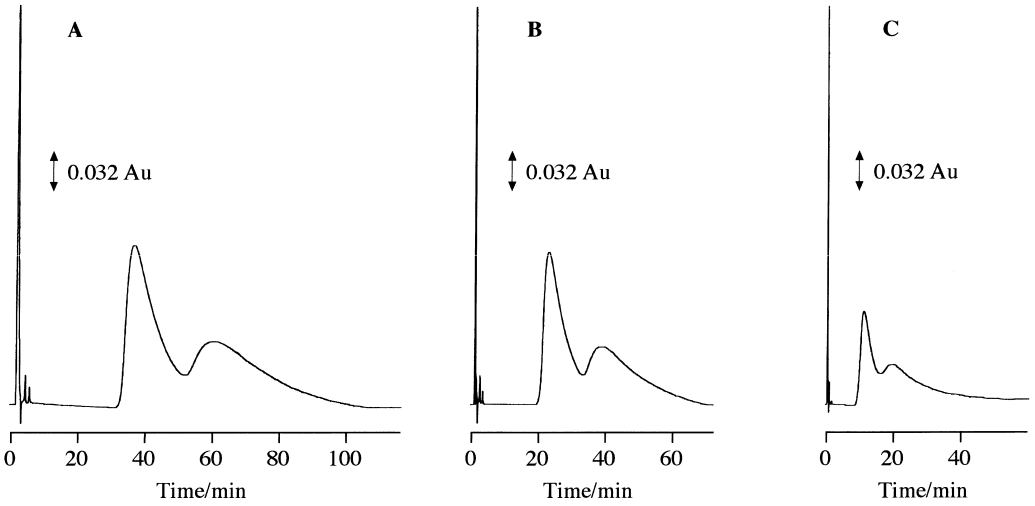

Fig. 5. Separation of chlorpheniramine enantiomers on the *d*-chlorpheniramine-imprinted MAA–EDMA columns at flow-rates of 0.5 (A), 0.8 (B) and 2.0 (C) ml/min. Key: 1, *R*-chlorpheniramine; 2, *d*-chlorpheniramine. HPLC conditions as in Table 6.

EDMA used were 2, 7 and 25 mmol, respectively, the enantioselectivity and resolution of chlorpheniramine was better despite the shorter retention times. With regard to the effects of column temperature and flow-rate, the column performance was improved by elevating the column temperature and decreasing the flow-rate.

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