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Direct enantioseparation of adrenergic drugs via thin-layer chromatography using molecularly imprinted polymers

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Abstract

Molecularly imprinted polymers (MIPs) of (−)-pseudoephedrine and (−)-norephedrine were prepared to use as chiral stationary phases (CSPs) in thin layer chromatography (TLC). The resolution of the enantiomers of adrenergic drugs, including pseudoephedrine, ephedrine, norephedrine, and epinephrine were investigated on these CSPs. In preparation of MIPs, two monomers: (1) methacrylic acid and (2) itaconic acid were employed as functional monomers. Mobile phase system of either methanol or acetonotrile was used and the effects of acetic acid content of the mobile phases were also investigated. The best resolution was achieved for enantioseparation of norephedrine on plates based on MIP of (−)-norephedrine using itaconic acid as functional monomer ($\alpha = 5.1$) in mobile phase 1% acetic acid in methanol. Moreover, these MIPs were able to resolve the racemates of compounds whose structures corresponded to print molecule. The results obtained showed that TLC based on MIPs could succeed the direct separation of enantiomers of adrenergic drugs as a method of separation. The method offers a rapid, sensitive and reliable method for quality control of optically active compounds.

Keywords: Enantioseparation; Thin-layer chromatography; Adrenergic drugs; Molecularly imprinted polymers; Chiral stationary phase; Enantiomers

1. Introduction

Phenylethanolamine adrenergic agonists such as pseudoephedrine, ephedrine, norephedrine and epinephrine are widely used for the treatment of nasal congestion, either alone or in combination. They possess a hydroxyl group on a chiral carbon (Fig. 1) which must be in the *R* absolute configuration for maximal direct activity as in the natural neurotransmitter. However, these drugs are currently marketed as mixtures of stereoisomers contained either *R* or *S* configuration at this chiral carbon [1]. The resolutions of these mixtures are therefore important, especially in commercial pro-

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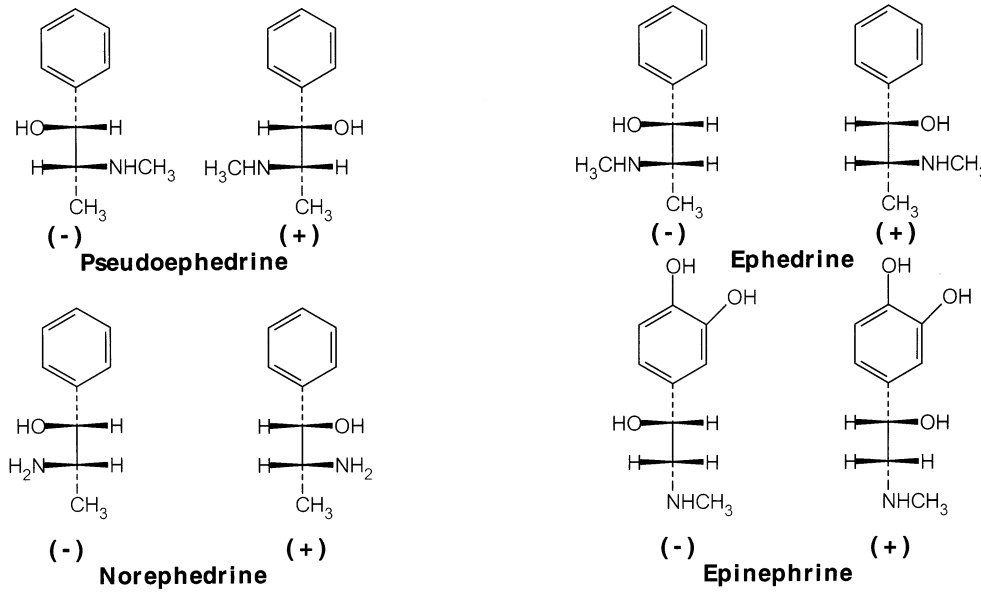

Fig. 1. Chemical structures of enantiomers of various adrenergic drugs studied.

duction and quality control. Enantioseparation of these drugs can be attained by indirect enantioseparation techniques which involve derivatizing the drugs with a chiral agent followed by separation by conventional chromatographic methods [2].

The aim of this study was to use molecularly imprinted polymers (MIPs) of adrenergic drugs as chiral stationary phases (CSPs) for direct enantioseparation of such compounds using thin-layer chromatography (TLC). MIP is particularly useful for separations of the chiral compounds [3,4] and immunoassay-like analyses involving either an antibody [5] or a receptor mimic [6]. MIPs have been prepared using diverse classes of compounds, such as amino acids and their derivatives [7–12], sugars [13,14], and a number of drugs [15–18]. There have been reports of successful separations of enantiomers by MIPs which obtained using chiral drugs such as the β-blocker, timolol [16]; the anti-inflammatory agent, naproxen [17]; and the anti-AIDs related drug, pentamide [18].

In general, the method of preparation of a MIP selective to chiral compound, involves the print molecule (or chiral template) being bound in a crosslinked polymer. The polymer is comprised of functional monomers and cross linking monomers. Thereafter, the chiral template is eliminated from the polymer and consequently specific recognition is left within the polymer network where arrangement of the functional groups of the polymer and shape corresponds to the template. The enantioselectivity of MIPs can be utilized by employing them as chiral stationary phases in both HPLC [19–22], TLC [23] and as mobile phase additives in capillary electrophoresis [24].

In our study the print molecules of (−)-pseudoephedrine and (−)-norephedrine were chosen for preparation of MIPs. The MIPs were then used as CSPs in TLC. Methacrylic acid which contains a carboxylic group capable of interacting via hydrogen bonding with a number of polar functionalities on a suitable print molecule, has been employed as a functional monomer in generating MIPs. Recently, Fischer et al. [16] employed a bifunctional monomer itaconic acid containing two adjacent carboxylic groups which showed greater potential for chiral separation than methacrylic acid in developing a MIP for HPLC chiral stationary phase. In this work, two

Table 1
Resolutions of adrenergic drugs on MIP of (−)-pseudoephedrine using methacrylic acid as functional monomer[a]

Drug	Acetic acid concentration in mobile phase (%)	Methanol			Acetonitrile		
		$R_f(-)$	$R_f(+)$	α	$R_f(-)$	$R_f(+)$	α
Norephedrine	0	0.07	0.11	1.6	0.08	0.08	1.0
	1	0.87	0.87	1.0	0.02	0.07	3.5
Epinephrine	0	0.21	0.21	1.0	0.43	0.43	1.0
	1	0.70	0.70	1.0	0.43	0.43	1.0
Pseudoephedrine	0	0.09	0.09	1.0	N	N	–
	1	0.84	0.84	1.0	0.14	0.16	1.1
Ephedrine	0	0.09	0.12	1.3	N	N	N
	1	0.94	0.94	1.0	0.13	0.16	1.1

[a] N, not detectable due to cracking of the plate. The R_f-values are averages of two determinations, the standard deviation being less than 0.02.

monomers methacrylic acid and itaconic acid were employed such that the acid group of the monomer interacts with amine and hydroxyl group of the print molecule. The effect of carboxylic groups of monomer used on the ability of polymers to separate enantiomers of adrenergic drugs was investigated as well as determining the stability of TLC plates made from these polymers. The resolution of the enantiomers of adrenergic drugs, including pseudoephedrine, ephedrine, norephedrine, and epinephrine (Fig. 1) were investigated. In addition, the importance of solvent comprised in the separation of products was examined by incorporating different concentrations of acetic acid in mobile phase systems of either methanol or acetonitrile.

2. Experimental

2.1. Chemicals and reagents

(+) and (−)-Pseudoephedrine, (±)-norephedrine, (−)-norephedrine, (±)-epinephrine and (−)-epinephrine were obtained from Aldrich (USA). (+)-Ephedrine was obtained from Sigma (St. Louis, MO). (±)-Ephedrine HCl was obtained from Fluka (Poole, Dorset, UK) and the free base was prepared by neutralizing with 1N NaOH. Ethylene glycol dimethacrylate, methacrylic acid and itaconic acid were purchased from Aldrich. 2,2′-Azobis-(2-methyl-propionitrile) (AIBN) was supplied from Janssen Chimica (Geel, Belgium). Anhydrous $CaSO_4$ was obtained from BDH (Poole, Dorset, UK). Other reagents were analytical grade or equivalent. All chemicals were used without further purification.

2.2. Preparation of molecularly imprinting polymers

Polymers were prepared using a method described previously [25]. All polymerization were performed under equivalent conditions and the composition of mixture was kept constant throughout experiments, except in the addition of print molecule. The polymerization reaction was carried out in sealed vials. A mixture of 5 mmol print molecule, 0.54 mol ethylene glycol dimethacrylate, 18 mmol methacrylic acid (or itaconic acid) and 1.5 mmol AIBN were dissolved in chloroform or tetrahydrofuran. Then, the mixture was degassed under vacuum in a sonicating water bath and sparged with nitrogen for 5 min before polymerization under UV light (366 nm) at 4°C for 18 h. Polymers were removed from vials and ground using a pestle and mortar. After drying the polymers were sieved (mesh size 100 μm) before sedimentation in acetonitrile under gravity. In order to remove print molecule, the polymers were kept in methanol/acetic acid (9:1, v/v) for 24 h followed by washing with acetonitrile and filtered. The absence of print molecule in the final

Table 2
Resolutions of adrenergic drugs on MIP of (−)-pseudoephedrine using itaconic acid as functional monomer[a]

Drug	Acetic acid concentration in mobile phase (%)	Methanol			Acetonitrile		
		$R_f(-)$	$R_f(+)$	α	$R_f(-)$	$R_f(+)$	α
Norephedrine	1	–	–	–	–	–	–
	5	0.32	0.32	1.0	0.18	0.18	1.0
	10	0.33	0.36	1.1	0.22	0.24	1.1
Epinephrine	1	0.26	0.38	1.5	–	–	–
	5	0.46	0.58	1.3	0.14	0.24	1.7
	10	0.56	0.64	1.1	0.44	0.54	1.2
Pseudoephedrine	1	0.28	0.30	1.1	0.10	0.10	1.0
	5	0.36	0.46	1.3	0.18	0.24	1.3
	10	0.40	0.50	1.3	0.20	0.29	1.5
Ephedrine	1	0.40	0.56	1.4	–	–	–
	5	0.44	0.56	1.3	0.28	0.38	1.4
	10	0.66	0.80	1.2	0.40	0.54	1.4

[a] The R_f-values are averages of two determinations, the standard deviation being less than 0.02.

Table 3
Resolutions of adrenergic drugs on MIP of (−)-norephedrine using methacrylic acid as functional monomer[a]

Drug	Acetic acid concentration in mobile phase (%)	Methanol			Acetonitrile		
		$R_f(-)$	$R_f(+)$	α	$R_f(-)$	$R_f(+)$	α
Norephedrine	0	0.05	0.22	4.4	0.03	0.14	4.7
	1	N	N	N	0.05	0.19	3.8
Epinephrine	0	0.42	0.52	1.2	0.06	0.11	1.8
	1	0.48	0.50	1.0	0.93	0.99	1.1
Pseudoephedrine	0	0.87	0.87	1.0	0.60	0.64	1.1
	1	0.89	0.89	1.0	0.90	0.90	1.1
Ephedrine	0	0.06	0.09	1.5	0.01	0.02	2.0
	1	0.87	0.89	1.0	N	N	N

[a] N, not detectable due to cracking of the plate. The R_f-values are averages of two determinations, the standard deviation being less than 0.02.

rinse, as determined by HPLC, verified maximum removal of print molecule from polymer. Polymers were finally dried under vacuum. Non-imprinted polymers (control) were prepared in the absence of print molecule. The particle size distribution of MIPs was determined by optical microscopy after grinding for 5 min. MIPs of the same batch were used throughout experiments.

2.3. Preparation of TLC plates

Each printed polymer (1 g) and anhydrous $CaSO_4$ (1 g) were gradually mixed with distilled water and a small amount of ethanol as wetting agent in a mechanical mortar. The slurry was carefully poured on standard glass microscope slides (76 × 26 mm), which was spread to form the thin layer with thickness 0.2 mm. The plates were dried at room temperature for at least 24 h. Control experiments were performed with the plates based on non-imprinted polymers.

2.4. Chromatographic method

All samples were dissolved in methanol at a concentration of approximately 1 mg ml^{-1} and

Table 4
Resolutions of adrenergic drugs on MIP of (−)-norephedrine using itaconic acid as functional monomer[a]

Drug	Acetic acid concentration in mobile phase (%)	Methanol			Acetonitrile		
		$R_f(-)$	$R_f(+)$	α	$R_f(-)$	$R_f(+)$	α
Norephedrine	0	0.08	0.41	5.1	0.12	0.24	2.0
	1	0.55	0.67	1.2	0.52	0.70	1.4
	5	0.66	0.74	1.1	0.38	0.50	1.3
	10	0.74	0.84	1.1	0.83	0.97	1.2
Epinephrine	0	0.04	0.12	3.0	–	–	–
	1	0.50	0.60	1.1	0.08	0.20	2.5
	5	0.68	0.82	1.2	0.30	0.40	1.3
	10	0.88	0.94	1.1	0.34	0.44	1.3
Pseudoephedrine	10	–	–	–	0.74	0.88	1.4
Ephedrine	0	0.18	0.28	1.6	–	–	–
	1	0.54	0.64	1.2	–	–	–
	5	0.60	0.70	1.2	0.32	0.44	1.4
	10	0.70	0.78	1.1	0.36	0.44	1.2

[a] The R_f-values are averages of two determinations, the standard deviation being less than 0.02.

carefully applied as spots at 1 cm above the bottom edge of the plate using 1-μl glass capillaries. For resolution studies, racemate was applied on the TLC plate along with an equal amount of individual enantiomer of the same drug. In the use of pseudoephedrine the pure enantiomers were applied separately. The chromatograms were developed with various concentrations of 0, 1, 5 and 10% (v/v) acetic acid in methanol or acetronitile. The plates were eluted to a distance approximately 5 cm from origin at ambient temperature.

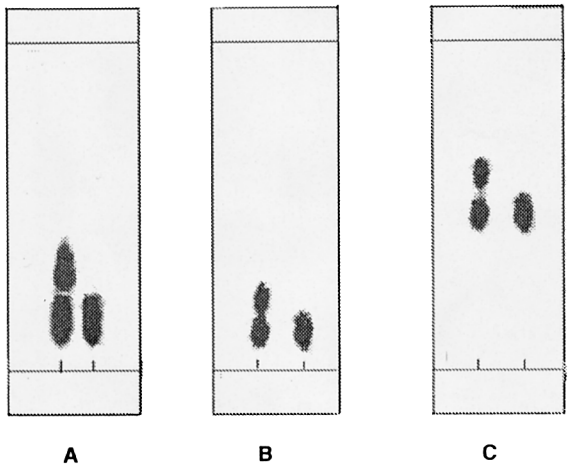

Fig. 2. Thin layer chromatograms of separations obtained for racemate (left) and (−)-enantiomer (right) of norephedrine on MIP (prepared from itaconic acid) of (−)-norephedrine: A developed with methanol; B developed with 1% acetic acid in acetonitrile; and C developed with 5% acetic acid in acetonitrile.

2.5. Visualization

The detection of the test samples on TLC plates was carried out after elution by spraying usually with ninhydrin reagent although for pseudoephedrine, acidified potassium permanganate reagent was employed. Heating of the plates was carried out as necessary to intensify reaction between the spray reagent and a sample. R_f-values were calculated; in the case of oval shaped or streaked spots the midpoint was taken. The chiral separation factor (α) of the two separated spots in the case of racemates applied was calculated as the ratio of the higher R_f-value and the lower R_f-value for the two enantiomers. Two determinations were made for all experiments.

3. Results and discussion

3.1. Preparation of MIPs

The MIPs based on (−)-pseudoephedrine and (−)-norephedrine using methacrylic acid and ita-

conic acid were successfully obtained. The preparation of MIP based on (−)-epinephrine was also attempted in this study but (−)-epinephrine poorly soluble in several organic solvents, MIP of this drug could not be accomplished. The amount of the remained print molecule was determined by FT-IR difference spectra between imprinted polymer and non-imprinted polymer, resulting no IR bands originating from print molecule could be detected (not shown). The attempted removal the entire print molecule from polymer is never completely successful and a small residual portion of the print molecule always remains in the polymer, this amount was not detectable by FT-IR. However, the portion remaining with the polymer must be minimized to avoid interference in spot detection on the TLC plate. It was found that MIPs of (−)-norephedrine always contained less residual print molecule than MIPs of (−)-pseudoephedrine, though the polymers were prepared under same conditions. The plates containing MIPs of (−)-norephedrine enabled the easiest detection of the test substances using either ninhydrine or potassium permanganate, since the spots clearly appeared in intense purple (or orange) on a white background.

3.2. Physical property and stabilty of the plates

It was difficult to prepare thin layer of MIP on glass support without binder as the crack formation of the TLC plate often occurred. The cracking of the plates was encountered when the amount of acetic acid in the mobile phase was increased (> 5%). It was found that the addition of $CaSO_4$ as a binder could improve the adhesion of MIP to the plate and improve the physical stability of stationary layer. The particle size of MIPs was examined to be in the range of particle size of 20–100 μm. A ratio of $CaSO_4$ to MIP of 1:1 was found suitable for MIPs of this particle size to enable preparation of TLC plates. It was found that the coating either formed cracks when smaller particle sizes (< 25 μm) were used or if the polymers were ground for longer than 5 min.

3.3. Enantiomeric resolution

Separation of adrenergic drugs on CSPs based on MIPs of (−)-pseudoephedrine. The resolution of adrenergic drugs on CSP based on MIP of methacrylic acid using (−)-pseudoephedrine as a print molecule and employing methanol and acetonitrile containing either 0 or 1% acetic acid are shown in Table 1. This CSP resolved the racemate of norephedrine into enantiomers (α = 3.5), although the R_f-values of both enantiomers were small, when 1% acetic acid was presented in acetonitrile, giving R_f-value of (+)-enantiomer higher than that of (−)-enantiomer. The chiral separation of pseudoephedrine was expected because this MIP was prepared using (−)-pseudoephedrine as the print molecule, but no elution of this drug occurred ($\alpha = 1.2$). No explanation can be given for the lack of separation. Furthermore, the other compounds failed to separate on this polymer. Control plates based on non-imprinted polymers of methacrylic acid did not give resolution for all compounds (data not shown), indicating that the polymers possessed no stereoselectivity for the enantiomers.

Separation data for adrenergic drugs on CSP based on MIP of (−)-pseudoephedrine using itaconic acid are given in Table 2. It can be observed that the overall difference between the R_f-values of (+)-isomer and (−)-isomer is sufficient to make distinction between the two enantiomers. Chiral separation factors between 1.2–1.7 were achieved for enantioseparation of the majority of compounds. Where resolution occurred, the (−)-enantiomer moved less than the (+)-enantiomer. In contrast to the polymer prepared from methacrylic acid, all spots failed to move from baseline in mobile phases in absence of acetic acid, denoting that the drugs adsorbed onto the polymer prepared from itaconic acid more strongly when less polar solvents were employed. This might be attributable in part to the two carboxylic groups of itaconic acid conferring a higher polarity on such a polymer. Even when at 1% acetic acid was included with either methanol or acetonitrile no spot movement of norephedrine enantiomers was observed.

The addition of acetic acid in mobile phases increased R_f-values (Table 2) and reduced the tail of spots as well as chiral separation factors in the most cases. The polymer of itaconic acid clearly demonstrates selectivity to more kinds of related compounds than the polymer prepared from methacrylic acid using same drug as print molecule, considering the effect of two adjacent carboxylic groups of itaconic acid used. Again, the plates based on non-imprinted polymers of itaconic acid showed no stereospecificity for all compound separations (data not shown). This confirms that chiral recognition of MIPs was contributed by incorporating a print molecule.

Separation of adrenergic drugs on CSPs based on MIPs of (−)-norephedrine. Table 3 shows the R_f-values and separation factors of adrenergic drugs which have been resolved on MIP of (−)-norephedrine using methacrylic acid as functional monomer with mobile phases containing either 0 or 1% acetic acid in methanol and acetonitrile. Norephedrine was efficiently resolved on this CSP in methanol ($\alpha = 4.4$) and acetonitrile ($\alpha = 4.7$). Enantiomers of most of the other compounds were satisfactorily resolved on this CSP. Employing 1% acetic acid content reduced the streaking of the substances on plate. R_f-values of (−)-norephedrine were always lower than those of (+)-norephedrine for all mobile phases, indicating greater affinity of the polymer prepared to (−)-norephedrine with the (−)-isomers.

Table 4 shows the resolution of adrenergic drugs on CSP based on MIP of (−)-norephedrine using itaconic acid. Like the polymer prepared from methacrylic acid, the chiral resolution occurred with this polymer for mobile phase systems of both methanol and acetonitrile. The best separation was achieved on this CSP for resolving the enantiomers of norephedrine in methanol ($\alpha = 5.1$, Fig. 2A). It can be seen in Tables 3 and 4 that the MIP prepared with methacrylic acid as monomer showed better separation of norephedrine racemate in acetonitrile, while the polymer prepared with itaconic acid better in methanol. In the use of acetonitrile as eluent, both enantiomers of norephedrine were retained on the MIP prepared from methacrylic acid more than on the MIP prepared from itaconic acid. It was observed that in acetonitrile, (−)-norephedrine greatly retained on polymer of methacrylic acid, giving rise higher separation factor for this polymer than polymer of itaconic acid. In methanol, the MIP prepared with itaconic acid gave R_f-value of (+)-norephedrine twice higher than that of the MIP prepared with methacrylic acid, while little difference of R_f-values of (−)-norephedrine was found between these polymers, resulting separation factor of the polymer prepared with itaconic acid was greater than that of polymer of methacrylic acid. These results suggest that the mobile phase can affect to retention of enantiomers, subsequently enantiomeric separation of these polymers.

Also, the MIP obtained from (−)-norephedrine enabled resolutions of the enantiomers of norephedrine when the acetonitrile contained 1 or 5% acetic acid ($\alpha = 1.4$ and 1.3, respectively) and spots were obtained without tailing as illustrated in Fig. 2B,C, respectively. Again, an increase in acetic acid content in mobile phases reduced the resolution of all compounds. Pseudoephedrine enantiomers did not move from baseline in any mobile phase except one of acetonitrile containing 10% acetic acid. Elution of ephedrine only occurred when the acetic acid content in mobile phases was 5% or more, no movement from the baseline occurring at lower acid concentrations.

The degree of selectivity of MIPs prepared to (−)-norephedrine using methacrylic acid and itaconic acid were not considerably different. However, in the case of itaconic acid, it was possible to perform elution in acetic acid contents as high as 10%. Further, the MIP prepared from itaconic acid exhibited a clear stereospecific trend as (−)-norephedrine more interacted strongly with the polymer than the (+)-norephedrine. The separation of epinephrine also occurred on this CSP in methanol ($\alpha = 3.0$) and in 1% acetic acid in acetonitrile ($\alpha = 2.5$). The (−)-epinephrine was retarded on this stationary phase more than (+)-epinephrine.

3.4. Chiral recognition of MIPs

The MIPs obtained in this study clearly showed potential for the chiral separation of adrenergic drugs by TLC. It was interesting to note that the

MIPs could be employed not only to resolve racemates of the same drugs as print molecules themselves but also enabled separation of some related chiral compounds. Here, the MIPs that imprinted with either (−)-pseudoephedrine or (−)-norephedrine, generally demonstrated an enantiomeric selectivity for the (−)-enantiomer of the compounds in comparison to the (+)-enantiomer. It would appear that the MIPs may also be enantiomerically selective for chiral compounds structurally related to the print molecule. All compounds contain a common hydroxyl substituted carbon (C_1) as asymmetric carbon which favored antipodes of the MIPs have *R* configuration at this position same as the print molecule. Therefore, the interaction of enantiomer and MIP may be at C_1. In this case, the *R* configuration of C_1 may play an important role in enantiomeric recognition of the MIP. Moreover, the structural differences between (−)-enantiomers of compounds studied were small; amine moiety was either primary amine or secondary amine (methyl substituted amine) which had different arrangements around the chiral carbon (C_2) at the end of the side chain, with the exception of one of epinephrine containing the absence of asymmetric arrangement at C_2 and two additional hydroxyl groups on benzene ring. MIPs prepared to either (−)-pseudoephedrine or (−)-norephedrine enable to distinguish these amino moieties, as enantioselectivity of the MIPs with various compounds were different, the best separation being obtained for the print molecule. These results demonstrate that the shape and the spatial arrangement of functional groups of the molecule are necessary for chiral recognition of MIP.

4. Conclusions

TLC remains an important laboratory technique for providing simple, rapid, reliable and inexpensive separation. Conditions for the direct resolution of the adrenergic drugs by TLC were identified in this studied using CSPs based on MIPs. Moreover, it would certainly be worthwhile attempting to apply MIPs to the resolution of other racemates and the work in this line is in progress. The use of MIPs as CSPs in TLC demonstrated that they may provide a potentially powerful tool for resolving chiral compounds and this is a useful method for quality control of optically active compounds. The use of other enantiomers as a print molecules to prepare MIPs is work that is currently in progress.

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