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Direct enantiomeric resolution of some cardiovascular agents using synthetic polymers imprinted with (–)-*S*-timolol as chiral stationary phase by thin layer chromatography

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Molecular imprinted polymers (MIPs) of *S*-timolol were prepared as chiral stationary phases (CSPs) in thin layer chromatography (TLC). The resolution of the enantiomers of some cardiovascular drugs, including propranolol, atenolol, timolol, nadolol, nifedipine and verapamil were investigated on these CSPs. A mobile phase system of either methanol or acetonitrile was used and the effects of acetic acid content of the mobile phases were also investigated. The best resolution was achieved for enantioseparation of propranolol, timolol and atenolol on plates based on MIP of (−)-*S*-timolol using methacrylic acid as functional monomer ($\alpha = 1.52$, 1.6, 1.59) respectively, using acetonitrile containing 5% acetic acid and ($\alpha = 1.47$, 1.52, 1.5) in methanol containing 1% acetic acid as mobile phases. The results obtained show that TLC based on MIPs could be applied in the direct separation of several β-adrenergic drugs. As the side chains on β−blockers are similar, it is possible that this method could also be used for the resolution of other racemates in this family of drugs. Racemic drugs structurally related to print molecule, were completely resolved into two spots with the MIP plates. In general the retention of (+)-*R*-isomers was greater than that of (−)-*S*-isomers, indicating lower stereoselectivity of the MIPs to the dextrorotatory isomers. The method offers a rapid, sensitive and reliable method for quality control for these drugs.

1. Introduction

Molecular imprinting technology is an attractive synthetic approach to mimic natural molecular recognition [1, 2]. Molecular imprinting is a method for the preparation of synthetic polymers with predetermined selectivity for various substances [3]. One approach of the technique involves noncovalent prearrangement of functional monomers in the presence of a print molecule prior to polymerization [4]. After the removal of the print molecules from the resulting macroporous polymer matrix, the resulting polymer contains recognition which due to their shape and the arrangement of the functional groups have affinity for the print molecules. A potential drawback for this technique is the fact that imprint material is required to be prepared in the initial step.

Since most chiral drugs on the market are currently administered as racemates, racemic resolution of drugs is a major potential application. β-Adrenergic blocking agents were chosen as a target for this study. The selectivity of the hybrid TLC was evaluated by elution of six clinically used β-adrenergic blockers. A TLC plate containing a non-imprinted polymer (i.e. one prepared in the absence of a template molecule; NIP) was also evaluated as a control.

The MIPs was then used as CSPs in TLC. The methacrylic acid which contains a carboxylic group capable of interacting via hydrogen bonding with several polar functionalities on a suitable print molecule, has been employed as a functional monomer in generating MIPs.

Molecular imprinting, a technique for preparing specific recognition sites in polymers [3–7] has previously been used successfully for TLC analysis of some amino acid derivatives, [8] adrenergic drugs, [9] and amino derivatives [10].

There are several advantages of the employment of MIP in chiral discrimination. Firstly, the enantiomeric order of elution is foreseen by predetermining of the enantiomer selected as the print molecule. A MIP permits molecular recognition for several types of compounds varying according to chiral template, whilst other chiral selectors have enantioselectivity only with certain types of compounds. MIP can be reused after removing the print molecule from such a polymer. The need to screen a range of CSPs to find one that affects a given separation can be dismissed when a MIP is used and subsequently the cost of analysis is reduced [11].

This paper describes the enantioseparation of several cardiovascular drugs namely propranolol, atenolol, timolol, nadolol, nifedipine and verapamil using TLC chiral stationary phases based on molecularly imprinted polymers (MIP) prepared using *S*-timolol as imprint molecule.

2. Investigations, results and discussion

Several solvent systems were examined as a chromatographic eluent, however, acetonitrile and methanol were found to be appropriate for elution-development of the cardiovascular drug used in this study. It is of interest to mention that the addition of acetic acid in the range of 1–10% resulted in the analytes being less retained and enabled better separation of the enantiomers as well as improving the spot shape.

Although imprinted polymer can be obtained either by photo-polymerization or thermal polymerization methods, the preparation of polymers in this study was based on photo-initiation method at low temperature (4 °C).

Control experiments were performed with plates prepared from nonimprinted polymers. The compounds tested were racemates of propranolol, timolol, nadolol, atenolol, nifedipine, and verapamil. All samples were dissolved in 70% ethanol at a concentration of 3 mg · ml ^{1} and applied 1 cm from the edge of the plate with 1-μl glass capillaries. The chromatograms were developed with mobile phases containing different concentrations of acetic acid (1, 5, 10% v/v) in either methanol or acetonitrile. Because of the fluorescent background of the thin layer plates, spots were

always visible under UV light ($\lambda = 254$ nm) and were identified by measurement and comparison of R_F values. The chiral separation factor α between two separated spots was calculated as the ratio of the higher R_f value and the lower R_f value for the two spots of the corresponding enantiomers.

The Table gives the retention and resolution data of three β-adrenergic drugs that were successfully resolved on CSP based on MIP of (−)-*S*-timolol, namely, propranolol, timolol, and atenolol, while the enantiomers of other drugs were not separated. The R_F values of (−)-isomers were lower than those of (+)-isomers, indicating greater affinity of the levorotatory (−)-isomers. Generally, for the enantiomerically separated drugs the α values were in the range of 1.1–1.7, with tailing of the spot up to 10.0 mm, such streaking occurred particularly when more polar mobile phases were used and may be due to a small population of recognition sites with very high affinity [12].

A 5% addition of acetic acid to acetonitrile as the mobile phase increased the R_f values of propranolol, timolol and atenolol.

It is apparent from the Table that the polymer from MAA with acetonitrile as mobile phase, including acetic acid in different percentage, caused the resolution of racemic propranolol, timolol and atenolol to its corresponding enantiomers. When 5% acetic acid in acetonitrile was used as mobile phase the chiral separation factors were 1.52, 1.6, 1.59 respectively, and when 1% acetic acid in methanol were used were 1.43, 1.52 and 1.5 respectively. Although the addition of acetic and in mobile phases increased R_f values, it reduced the tailing of spots as well as chiral separation factors in most cases. This may be due to the competition of acetic acid with the analyte for the binding sites on the MIPs.

No enantiomeric separation was observed for racemic nadolol, nifedipine and verapamil on this CSP.

The *S*-timolol imprinted MIP was enantiomerically selective for chiral compounds structurally related to the print molecule. This is probably due to the similarity of the side chains that contain the chiral centre within this class of compounds. MIP 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 [7, 8] such as propranolol and atenolol. In these resolutions, the R_f values of (−)-*S*-enantiomers were lower than those of (+)-*R*-enantiomers, indicating greater affinity of the (−)-*S*-enantiomers to the stationary phase.

No separation and much less retention was observed on the nonimprinted plate. As expected, the retention on the reference plate was less than on the plates imprinted with pure S-timolol, since less recognition sites were available for each enantiomers.

All resolved compounds have the same chiral asymmetric carbon as the print molecule, while the chiral position of the unresolved compounds possess different spatial arrangement of functional groups and molecular size to fit in MIP which is necessary for chiral recognition.

In conclusion, the use of MIPs as CSPs in TLC demonstrated that they may give a powerful tool for resolving chiral compounds and becomes useful for quality control of optically active compounds. MIPs could be employed not only to resolve racemates of the same drug as print molecules themselves but also enabled separation of some structurally related chiral compounds. Accordingly, MIPs could be considered enantiomerically selective for resolution of racemic chiral compounds structurally related to the print molecule used.

Table: Chromatographic data on the separation of some β-adrenergic drugs on TLC plates containing a stationary phase based on imprinted polymers (*S*-timolol)

Compd.	Monomer	Acetic acid conc. in mobile phase	Methanol		α	Acetonitrile		α
			R_{f_1}	R_{f_2}		R_{f_1}	R_{f_2}	
Propranolol	MAA	1	28	18	1.47	24	22	1.1
		5	35	34	1.03	44	29	1.52
		10	37	34	1.1	51	46	1.1
Timolol	MAA	1	38	25	1.52	25	23	1.1
		5	36	29	1.24	45	28	1.6
		10	52	46	1.13	44	38	1.15
Atenolol	MAA	1	36	24	1.50	23	20	1.13
		5	51	42	1.21	46	29	1.59
		10	52	48	1.1	56	49	1.15

R_{f_1}: First eluted enantiomer
R_{f_2}: Second eluted enantiomer
α: R_{f_1}/R_{f_2}

3. Experimental

3.1. Materials

Methacrylic acid (MAA) and ethylene glycol dimethacrylate (EDMA) were obtained from Aldrich (Milwaukee, WI, USA). 2,2′-Azobis (butyronitrile) (AIBN) was purchased from Riedel-De Haen AG (Seelze-Hannover, Germany), (+)-*R*-propranolol, (−)-*S*-propranolol, racemic propranolol HCl, (+)-*R*-atenolol, (−)-*S*-atenolol, racemic atenolol HCl, (+)-*R*-nadolol, (−)-*S*-nadolol, racemic nadolol, (+)-*R*-nifedipine, (−)-*S*-nifedipine, racemic nifedipine, (−)-*S*-verapamil, (+)-*R*-verapamil and racemic verapamil HCl were obtained from Winlab (Leicestershire, LE16 9EJ, UK). (−)-*S*-Timolol, (+)-*R*-timolol, and racemic timolol were purchased from Sigma (St. Louis, MO, USA). Silica gel 60 GF_{254} was obtained from Merck (Darmstadt, Germany). All other reagents were analytical grade or equivalent. All chemicals were used without further purification.

3.2. Preparation of the molecular imprinted polymers

The preparation procedure of polymers was similar to that outlined by Meng et al. [12] and O'Shannessy et al. [13] Methacrylic acid and ethyleneglycol dimethacrylate (EDMA) were used as functional monomer and cross-linking monomer, respectively. S-Timolol (0.5 mmol) was dissolved in tetrahydrofuran. Methacrylic acid (18 mmol), EDMA (0.54 mol), and the initiator AIBN (0.25 mmol) were added. The mixture was then degassed under vacuum in a sonicating water bath and purged with nitrogen for 5 min. The polymerization was carried out under UV light ($\lambda = 366$ nm) at 4 °C for 24 h. The resulting polymer was ground and sieved through a 100-μm sieve. To remove imprint molecule, the polymer was immersed in 10% acetic acid in acetonitrile for 24 h, washed with acetonitrile, and dried overnight under vacuum. Non-imprinted polymers used as the control were prepared similarly but in the absence of the print molecule namely (−)-*S*-timolol.

4.3 Preparation of thin-layer chromatography plates

Each polymer (100 mg) and silica gel 6 GF_{254} (100 mg) were gradually mixed with distilled water (1.4 ml) and a small amount of ethanol (10 μl), as wetting agents, using a pestle and a mortar. The slurry was poured on standard glass microscope slides (76 × 26 mm), which then spread as a thin layer, with a thickness of about 0.25 mm, and dried for at least 24 h at room temperature. It was found that the addition of $CaSO_4$ (included in the silica gel 60 GF_{254}, as 13%) as a binder could improve the adhesion of MIP to the plate and improve the physical stability of stationary layer [11].

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