Short Communication

Direct resolution of naproxen on a non-covalently molecularly imprinted chiral stationary phase ☆

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Abstract

A synthetic polymer selective for (*S*)-naproxen was prepared by molecular imprinting. 4-Vinylpyridine and ethylene glycol dimethacrylate were copolymerised in the presence of the template, (*S*)-naproxen. The template was extracted from the polymer, leaving specific recognition sites, complementary to the template. The polymer was utilized as a stationary phase in HPLC. Racemic naproxen was efficiently resolved on the polymer. Furthermore, the polymer was able to separate naproxen from the structurally related ibuprofen and ketoprofen.

1. Introduction

Naproxen (6-methoxy-α-methyl-2-naphtylene-acetic acid) (**1**, Fig. 1) is a 2-arylpropionic acid non-steroidal anti-inflammatory drug (2-APA-NSAID). Drugs of this class, the majority of which possess a chiral centre, are generally administered as their racemates, with the excep-
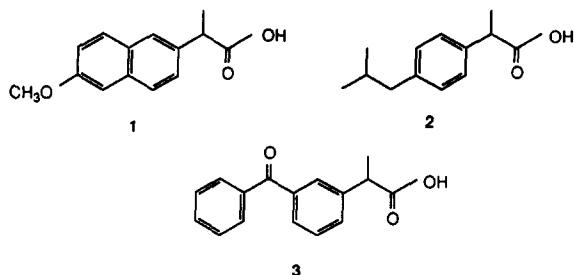

Fig. 1. Structures of the studied compounds, (**1**) naproxen, (**2**) ibuprofen and (**3**) ketoprofen.

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tion of naproxen, which is administered as its *S* enantiomer. This has stimulated extensive interest in the resolution of racemic 2-APA-NSAIDs, especially in the areas of quality control analysis [1] and pharmacokinetic studies [2]. This is reflected by the number of chromatographic studies that has been published, including direct resolution on chiral stationary phases (CSPs) [1,3–7], derivatization with chiral/non-chiral reagents followed by resolution on non-chiral/chiral stationary phases [1,8–12] and direct resolution on non-chiral stationary phases using chiral mobile phase additives [13]. The acidic nature of NSAIDs precludes the use of most of the commercially available HPLC CSPs for direct resolution [3].

This communication describes, to the best of our knowledge, the first report of the preparation of a CSP tailor-made for the enantioseparation of naproxen. The CSP is prepared using non-covalent molecular imprinting, sometimes referred to as template polymerisation, which is

a technique for preparing recognition sites of predetermined specificity in synthetic polymers. This technique entails the prearrangement of functional monomers with the molecule of interest, the template (synonymous definition used is print molecule). After copolymerisation of functional monomers and cross-linker, the template is removed from the polymer by extraction, resulting in a polymer with specific recognition sites, complementary to the template in the positioning of the functional groups and in the shape. Due to the cognitive properties of the polymer, it is able to selectively rebind the template species [14–16]. Molecular imprinting has previously been applied in optical resolutions of amino acid derivatives [17–24] and β-blockers [25].

2. Experimental

2.1. Chemicals

(R)-Naproxen was a gift from Syntex Nordica (Södertälje, Sweden). (S)-Naproxen, ibuprofen and ketoprofen were from Sigma (St. Louis, MO, USA). 4-Vinylpyridine, ethylene glycol dimethacrylate and 2,2'-azobis(2-methylpropionitrile) were purchased from Merck–Schuchardt (Germany). All organic solvents were of analytical or HPLC grades.

2.2. Equipment

The bulk polymers were ground in a Retsch end runner mill Model RM O (Haan, Germany). A 25-μm Retsch sieve was used for particle sizing. The HPLC analyses were performed using a Kontron HPLC system comprising a pump 420, a gradient former 425 and a variable-wavelength detector 432. The column was packed using an air-driven fluid pump from Haskel Engineering (Burbank, CA, USA).

2.3. Preparation of the polymer

A 0.46-g amount of (S)-naproxen (2 mmol), 1.26 g 4-vinylpyridine (12 mmol), 11.89 g ethyl-

ene glycol dimethacrylate (60 mmol) and 0.115 g 2,2'-azobis(2-methylpropionitrile) (0.7 mmol) were dissolved in tetrahydrofuran (THF) (18 ml). The mixture was sonicated and deoxygenated with a stream of nitrogen, then irradiated with UV light (366 nm) at 4°C for 48 h. The bulk polymer was ground in a mechanical mortar and wet-sieved by hand with water and ethanol through a 25-μm sieve. The particles which passed the sieve were collected, dried on a sintered glass funnel and allowed to sediment (5 × 20 min) in acetonitrile (300 ml). The particles that did not sediment were discharged.

2.4. High-performance liquid chromatography

The sieved and sedimented polymer particles were packed at 300 bar into a stainless-steel HPLC column (200 × 4.6 mm) using acetonitrile as solvent. After packing, the column was eluted with THF–acetic acid (7:3, v/v) at 1 ml/min until a stable baseline was achieved. The eluent used for the separation studies was THF–heptane–acetic acid (250:250:1, v/v/v). The flow-rate was 0.1 ml/min, the elution was monitored at 260 nm and the separation was performed at ambient temperature.

The separation factor (α) was determined using the relationship $\alpha = k'_S/k'_R$, where k'_S is the capacity factor of the S enantiomer and k'_R is the capacity factor of the R enantiomer. The capacity factors were determined according to $k'_S = (t_S - t_0)/t_0$, where t_S is the retention time of the S enantiomer and t_0 is the retention time of the void, which was determined by injection of toluene. The resolution factor was determined according to Meyer [26].

3. Results and discussion

The aim of this study was to use molecular imprinting for the preparation of a synthetic polymer selective for naproxen. The clinically useful (S)-naproxen was used as template molecule. 4-Vinylpyridine was chosen as the functional monomer, on the basis that it has previously been shown to be efficient in the prepara-

Table 1
Resolution of naproxen on the molecularly imprinted polymer

Loaded amount of (R,S)-naproxen (μg)	k'_R	k'_S	α	R_s
2	2.17	3.58	1.65	0.83
20	1.74	2.20	1.26	0.69

tion of molecularly imprinted polymers selective for N-protected amino acids [22]. It is assumed that 4-vinylpyridine interacts with the carboxy group in naproxen by ionic interactions. (R,S)-Naproxen was well resolved on this CSP, as can be seen in Table 1 and Fig. 2. The flow-rate was 0.1 ml/min. Higher flow-rates resulted in less resolved peaks. A separation factor of 1.65 and a resolution factor of 0.83 were obtained when 2 μg of the racemate was loaded on the column (Fig. 2). When the loaded amount was increased to 20 μg, the separation factor was 1.26 and the resolution factor was 0.69. These values compare well with those previously reported for direct resolution of naproxen on conventional CSPs ($\alpha = 1.71$ [1], 1.32 [3] and 1.31 [7]).

The imprinting procedure gives rise to specific recognition sites in the polymer. It was of interest to investigate if this CSP, designed specifically for naproxen, was able to resolve other 2-APA-NSAIDs. T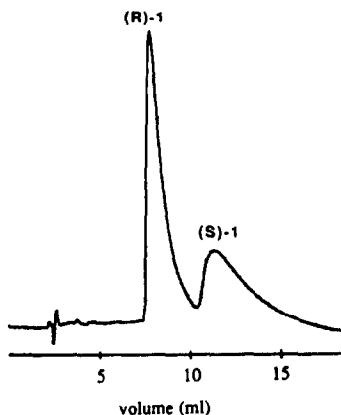he methyl and the carboxy groups attached to the chiral carbon are common to all 2-APA-NSAIDs, though the aryl-substituents vary. According to Dalgliesh [27], a "three-point" interaction is necessary for stereochemical specificity. Therefore, it is required that not only the carboxy group attached to the chiral carbon interacts with the imprinted polymer, as discussed above, but also the methyl and aryl substituents. The role of the aryl substituent in the recognition mechanism was elucidated by investigation of whether the polymer was able to resolve related 2-APA-NSAIDs. It was shown that neither the racemate of ibuprofen (2, Fig. 1) nor ketoprofen (3) were resolved on this CSP. Accordingly, the shape, size and nature of the aryl substituent is important for the recognition under the conditions chosen.

We were also interested to see if this molecularly imprinted stationary phase was able to separate a mixture made up of the optical antipodes of ibuprofen, ketoprofen and naproxen. As can be seen in Fig. 3, (R,S)-ibuprofen and (R,S)-ketoprofen were both eluted as single peaks, whereas (R)- and (S)-naproxen were clearly resolved. The polymer may therefore be used as a stationary phase also for separation of various 2-APA-NSAIDs.

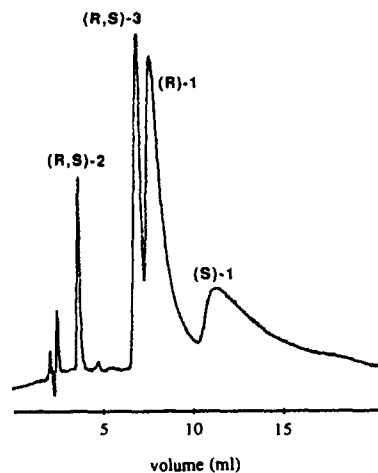We believe that this tailor-made polymer,

Fig. 2. Chromatographic resolution of 2 μg (R,S)-naproxen on the naproxen-imprinted polymer.

Fig. 3. Separation of a mixture of ibuprofen (2 μg), ketoprofen (0.2 μg) and naproxen (2 μg) on the naproxen-imprinted polymer. Only naproxen was resolved into its enantiomers ($\alpha = 1.70$).

specific for the determination of naproxen, represents, due to its excellent chemical and physical stability, a valuable alternative to existing CSPs utilizing biomolecules, such as bovine serum albumin, α_1-acid glycoprotein or ovomucoid. In this context, it should be mentioned that the polymer investigated here was used over 80 times without any decrease in performance. We are currently investigating the preparation of molecularly imprinted polymers selective for other NSAIDs.

4. Acknowledgement

5. References

[1] J.R. Kern, *J. Chromatogr.*, 543 (1991) 355.
[2] W.J. Wechter, D.G. Loughhead, R.J. Reischer, G.J. Van Giessen and D.G. Kaiser, *Biochem. Biophys. Res. Commun.*, 61 (1974) 833.
[3] T.A.G. Noctor, G. Felix and I.W. Wainer, *Chromatographia*, 31 (1991) 55.
[4] T. Miwa, T. Miyakawa, M. Kayano and Y. Miyake, *J. Chromatogr.*, 408 (1987) 316.
[5] J. Hermansson and M. Eriksson, *J. Liq. Chromatogr.*, 9 (1986) 621.
[6] Y. Okamoto, R. Aburatani, Y. Kaida, K. Hatada, N. Inotsume and M. Nakano, *Chirality*, 1 (1989) 239.
[7] S. Allenmark and S. Andersson, *Chirality*, 1 (1992) 24.
[8] I.W. Wainer and T.D. Doyle, *J. Chromatogr.*, 284 (1984) 117.
[9] D.A. Nicoll-Griffith, *J. Chromatogr.*, 402 (1987) 179.
[10] D.A. Nicoll-Griffith, T. Inaba, B.K. Tang and W. Kalow, *J. Chromatogr.*, 428 (1988) 103.
[11] D.M. McDaniel and B.G. Snider, *J. Chromatogr.*, 404 (1987) 123.
[12] R. Dernoncour and R. Azerad, *J. Chromatogr.*, 410 (1987) 355.
[13] C. Pettersson and K. No, *J. Chromatogr.*, 282 (1983) 671.
[14] R. Arshady and K. Mosbach, *Makromol. Chem.*, 182 (1981) 687.
[15] B. Ekberg and K. Mosbach, *Tibtech.*, 7 (1989) 92.
[16] G. Wulff, *Am. Chem. Soc. Symp. Ser.*, 308 (1986) 186.
[17] L. Andersson, B. Sellergren and K. Mosbach, *Tetrahedron Lett.*, 25 (1984) 5211.
[18] B. Sellergren, M. Lepistö and K. Mosbach, *J. Am. Chem. Soc.*, 110 (1988) 5853.
[19] D.J. O'Shannessy, B. Ekberg and K. Mosbach, *Anal. Biochem.*, (1989) 144.
[20] L.I. Andersson and K. Mosbach, *J. Chromatogr.*, 516 (1990) 313.
[21] M. Kempe and K. Mosbach, *Anal. Lett.*, 24 (1991) 1137.
[22] M. Kempe, L. Fischer and K. Mosbach, *J. Mol. Recogn.*, 6 (1993) 25.
[23] M. Kempe and K. Mosbach, in preparation.
[24] O. Ramström, L.I. Andersson and K. Mosbach, in press.
[25] L. Fischer, R. Müller, B. Ekberg and K. Mosbach, *J. Am. Chem. Soc.*, 113 (1991) 9358.
[26] V.R. Meyer, *Chromatographia*, 24 (1987) 639.
[27] C.E. Dalgliesh, *J. Chem. Soc.*, 137 (1952) 3940.