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Short communication

Optimisation of procedures for the extraction of structural analogues of propranolol with molecular imprinted polymers for sample preparation

Paul Martin[a,*], Ian D. Wilson[a], Graeme R. Jones[b]

[a]*Department of Safety of Medicines, AstraZeneca UK Ltd., Mereside, Alderley Park, Macclesfield, Cheshire SK10 4TG, UK*
[b]*Department of Chemistry, University of Keele, Keele, Staffordshire UK ST5 5BG, UK*

Abstract

A propranolol-derived molecular imprinted polymer (MIP) was prepared using methacrylic acid as monomer and ethylene glycol dimethacrylate as cross-linker. The extraction properties of five compounds structurally related to propranolol were assessed on the MIP and on a blank polymer made under the same conditions but in the absence of an imprint molecule. Using application from aqueous solution with methanol–water–triethylamine (TEA)-based solvents for elution (i.e. reversed-phase conditions) the MIP showed only marginal selectivity for the compounds on the MIP compared to the blank. Despite the limited selectivity there did appear to be a relationship between structure of the compound (relative to propranolol) and the extent of selective retention. Application of the compounds in toluene with elution using toluene–TEA or toluene–trifluoroacetic acid resulted in the MIP showing dramatically enhanced retention and selectivity of the compounds on the MIP compared to the blank. The enhanced selectivity for extraction on to the MIP relative to the blank, for all compounds using normal-phase solvents seem to be a class effect as there was no apparent relationship between compound structure and retention.

Keywords: Molecular imprinting; Extraction methods; Propranolol; β-Blockers

1. Introduction

Molecularly imprinted polymers (MIPs) have been used as solid-phase extraction (SPE) materials in a number of methods to extract a range of structurally diverse compounds. Reviews of published work [1–3] reveal that MIPs have been used in sample preparation in a variety of different formats including conventional SPE cartridges as well as in-line extraction devices prior to high-performance liquid chromatography (HPLC). The application and elution solvents vary between methods and the choice is partly driven by the nature of the analyte. Work within this laboratory [4] using a propranolol-derived MIP illustrated that selective elution conditions can be found with methanol–water-based solvents containing triethylamine (TEA) whereas trifluoroacetic acid (TFA)-containing solvents were not selective. Both the acidic and basic modifiers were used to overcome ionic interactions between the basic analyte and the acidic MIP but TEA achieved this in a manner consistent with retention due to a mechanism based on molecular imprinting. While this trend appeared promising, when structurally diverse compounds were extracted, the results with structural analogues of propranolol showed a degree of ''cross reactivity'' to close analogues [5]. In addition, some molecular imprints, although prepared under the

*Corresponding author. Fax: +44-1625-583-074.

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same conditions as selective polymers, showed negligible selectivity in extraction for propranolol and its analogues. Indeed in some case the elution curves on the imprinted polymer were not significantly different to those on a blank polymer. Because of these observations we concluded that the application and elution solvents for these MIPs in SPE had not been optimised to exploit interactions due to imprinting. The majority of the experiments performed involved the application of aqueous samples, which is consistent with the widest application of SPE methods and we sought to retain this in the methods developed. However, it is widely recognised that the best interactions of the imprint molecule and MIP are likely to occur under similar conditions of solvent, etc., to those used to prepare the polymer. The aim of the work described here was to investigate alternative application and elution conditions designed to maximise these selective extractions for a propranolol-derived molecular imprint.

2. Experimental

2.1. Materials

A propranolol-imprinted polymer and a blank (non-imprinted) polymer were used in this investigation. The polymers were prepared according to the method of Andersson [7] using methacrylic acid as the monomer and ethylene glycol dimethacrylate as the cross-linker. Racemic propranolol (Sigma, Poole, UK) was used as the template molecule. A molar ratio of 2:1 methacrylic acid–propranolol was used to prepare the MIP. The imprinted and blank polymers were prepared in toluene with the polymerisation initiated by heating at 60°C for 18 h.

Following polymerisation, the solid polymers were ground using a pestle and mortar and sieved through a 50-μm sieve. The particles were washed with methanol–acetic acid (3:1) (three times) and finally sedimented in methanol three times to remove fines. The sedimented polymers were recovered and dried in a vacuum oven at 60°C.

Five compounds (see Fig. 1 for structures) structurally related to propranolol (also Fig. 1) were used to evaluate the polymers for extraction and structural selectivity. These were M109056, M52487, M47070, M51932 and M115716 and were all supplied by AstraZeneca Pharmaceuticals.

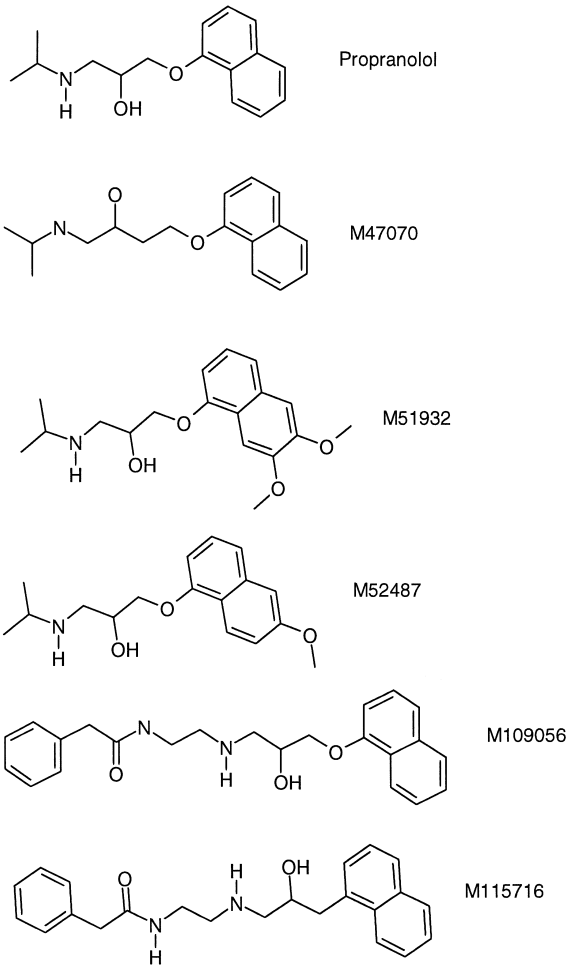

Fig. 1. Structures of propranolol, M109056, M52487, M47070, M51932 and M115716.

2.2. Procedures

Extraction cartridges, 1-ml reservoirs (IST, Hengoed, UK) were packed with 30 mg of the propranolol-imprinted polymers or the blank polymer. Two different extraction protocols were employed:

Protocol 1: Cartridges were solvated with methanol (1 ml) and water (1 ml). The aqueous sample (0.5 ml) was applied (containing each compound at a concentration of 10 μg/ml), followed by a water wash (0.5 ml). Serial elution was performed using

0.5-ml aliquots of a range of methanol–water-based solvents (containing 10, 20, 30, 40, 50, 60, 70, 80, 90, 100% methanol). An ionic modifier (either TEA or TFA) was present in each solvent at a concentration of 1%.

Protocol 2: Cartridges were solvated with toluene (2 ml). The analytes in toluene (0.5 ml) (at a concentration of 10 μg/ml) were then applied, followed by a toluene wash (0.5 ml). Serial elution was performed using toluene (0.5 ml) containing either TEA or TFA at the following percentages: 0.00195, 0.0039, 0.0078, 0.0156, 0.0312, 0.0625, 0.125, 0.25, 0.5, 1, 2% (v/v).

The eluents from each application, wash and elution step were individually reduced to dryness under a stream of oxygen-free nitrogen at 30°C. The residues were re-dissolved in mobile phase (400 μl) and aliquots (50 μl) injected onto the HPLC system for quantification. The HPLC system consisted of a HiRPB column (25 cm×4.6 mm, 5 μm) supplied by Hichrom. The mobile phase was methanol–water–TFA–ammonium acetate (550:450:1:7.7, v/v/v/w) at 1 ml/min with detection by UV at 230 nm using a Perkin-Elmer LC 290 detector. The mobile phase was delivered by an LDC Analytical Constametric 3200 pump (Stoke, UK).

3. Results and discussion

Previous investigations using propranolol-derived MIPs in this laboratory revealed that selective extraction of propranolol and close structural analogs was possible but problems were encountered with HPLC analysis of extracted propranolol because of leaching of the template [5,6]. This was seen as a significant limitation of the MIP for the analysis of the imprint molecule. However, in the current experiments we have used close structural analogs of propranolol because we believe that we will be able to develop analytical methods for these compounds using imprint-based extractions without template leaching causing problems.

The initial experiment involved application of the five compounds (in water) to the MIP and the blank polymer, followed by cumulative elution in methanol–water–TEA solvents (protocol 1) which had previously been shown to provide the greatest selectivity for propranolol-derived MIPs [4]. The cumulative elution curves for each compound on the MIP compared to the blank polymer are illustrated in Fig. 2A (MIP) and Fig. 2B (blank). In general the curves for compounds on the MIP were shifted somewhat to the right relative to the blank, indicating greater retention on this MIP. However, the magnitude of these differences are not overly impressive. The observed differences in elution profiles can be rationalised to some extent with reference to the structures of the compounds compared to the imprint molecule. Thus, M47070, the most selectively retained compound on the MIP compared to the blank, behaves in essentially the same way as propranolol.

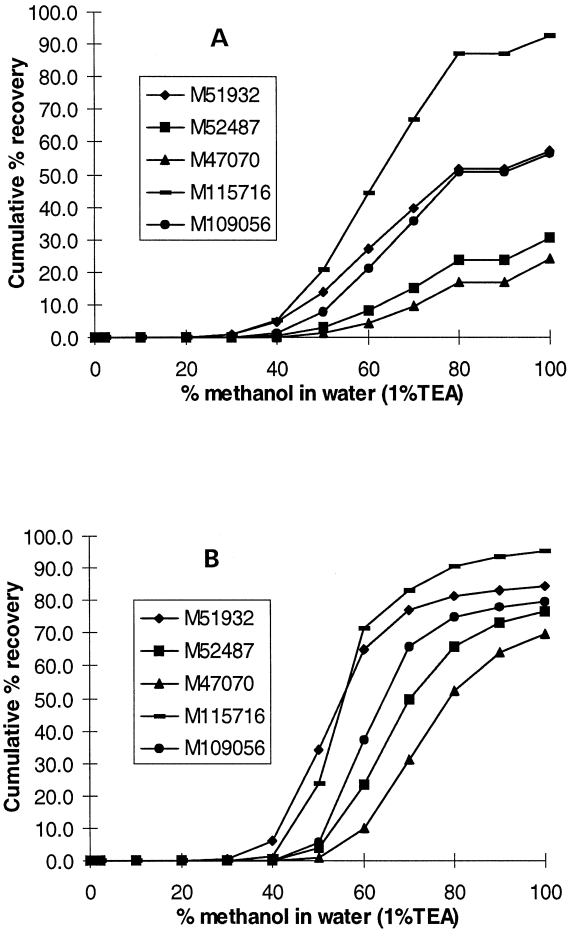

Fig. 2. Cumulative percentage recovery of M109056, M52487, M47070, M51932 and M115716 using methanol–water–TEA from (A) the MIP and (B) the blank polymer.

This compound is the closest in structure to propranolol, differing only in having an additional carbon atom in the side chain. The compounds M52487 and M51032 showed moderate differences between MIP and blank and these compounds differed from propranolol in having either one or two additional *O*-methyl groups, respectively bonded to the fused rings. These additions seemingly do not interfere significantly with the imprint-based interactions which are presumably dominated by interactions with the side chain. The remaining two compounds M115716 and M109056 showed similar elution curves on the MIP and blank indicating that retention was primarily due to non-specific binding and imprint-based binding played a negligible role. These two compounds were quite different from propranolol in that both were amides and possessed longer side chains than propranolol. Overall the relatively small differences in the elution curves between MIP and blank indicated that non-specific binding played a considerable role in retention on the MIP compared to selective imprint-based binding. The small contribution of selective imprint-based retention would probably mean that it would be difficult to establish a selective analytical method on this MIP which depended on imprint-based binding using methanol–water elution solvents.

In order to overcome this problem we investigated the properties of the MIP with organic solvents for application and elution in the hope that greater selectivity could be achieved. This approach was based on literature examples (e.g., Ref. [7]) where MIPs have shown better molecular recognition in organic solvents than in aqueous systems. We evaluated the retention of the analytes when applied in toluene, which was the solvent used in the polymerisation process. For this experiment the polymers (MIP and blank) were conditioned in toluene and the compounds were applied in toluene (protocol 2). In order to produce cumulative elution curves it was necessary to either mix the toluene with a more eluotropic solvent or to increase the strength of the ionic modifier in the toluene. We decided to use toluene and vary the concentration of the ionic modifier (TEA or TFA) by doubling the acid or base concentration in successive eluents. The elution curves for M115716 on the MIP and blank polymer using toluene–TEA and toluene–TFA eluents are

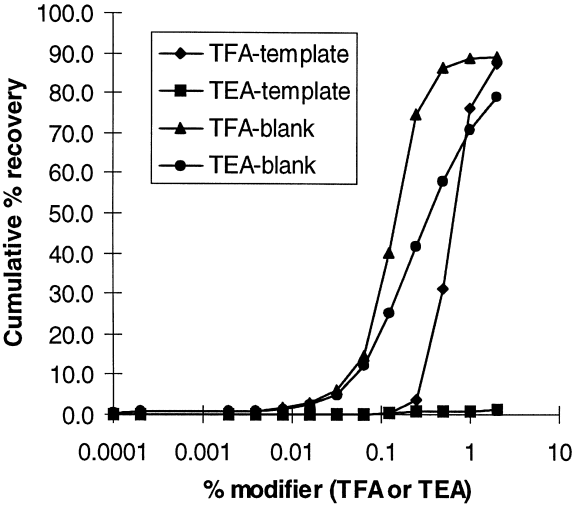

Fig. 3. Cumulative percentage recovery of M115716 using toluene–TEA and toluene–TFA from the MIP and blank polymer.

illustrated in Fig. 3. These results were typical of those seen for all of the test compounds and indeed it was difficult to see any differences between the curves for each compound. Comparison of the toluene–TFA elution curves indicated that the imprinted polymer demonstrates somewhat more selective retention compared to the blank with the elution curve shifted to the right but still similar in shape. For the TEA-containing eluents the curve on the MIP was shifted markedly to the right compared to the blank polymer and even 2% TEA only recovered approximately 2% of the compound from the MIP. It was also noteworthy that the elution curves using toluene–TEA and toluene–TFA were similar on the blank polymer but that the toluene–TEA were significantly shifted to the right compared to toluene–TFA on the MIP. Comparison of the results for the blank and MIP, using toluene based as opposed to methanol–water-containing solvents, demonstrated that much greater selectivity could be achieved using a MIP in organic rather than aqueous solvents. In addition, these experiments showed that while this selectivity was much better with TEA as the ionic modifier than TFA it was still possible to achieve some selectivity with TFA-containing solvent. This effect was not our experience using aqueous solvent systems for elution [4]. Although these findings were promising it was evident that

while some structure–retention relationship was evident for extraction and elution using aqueous solvents no obvious relationship was evident using normal-phase solvents. However, it is not difficult to envisage a use for a ''class specific'' MIP as well as a highly specific imprinted polymer.

4. Conclusions

These experiments demonstrate that efficient extractions of the test compounds, with highly selective retention and elution, could be achieved on a propranolol-derived MIP with organic solvents where aqueous systems gave poor results. Although the MIP showed better retention of the compounds than the blank polymer there was no apparent structure–retention relationship with compounds quite different to propranolol showing similar selectivity between MIP and blank as propranolol itself. The evident selectivity between MIP and blank in normal-phase solvents, whilst expected, might represent a serious limitation of the technique in SPE where the majority of biological samples for extraction are aqueous in nature. If the samples first had to be extracted into an organic solvent and only then be applied to the MIP the added complication of the method would significantly reduce its attractiveness compared to alternative methodologies. To attempt to overcome this limitation we are currently investigating protocols that allow the application of aqueous samples to the cartridge but then exploit the enhanced selectivity that can be achieved using organic solvents for elution.

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