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Comparison of extraction of a β-blocker from plasma onto a molecularly imprinted polymer with liquid–liquid extraction and solid phase extraction methods

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Abstract

An optimised solid phase extraction (SPE) method developed for the extraction of a structural analogue of the β-blocking drug propranolol from plasma utilising a molecularly imprinted polymer (MIP) has been compared with methods based on conventional liquid–liquid extraction (LLE), and SPE using C18-bonded and immobilised phenyl boronic acid (PBA). All four methods could be used for the extraction of the analyte with acceptable accuracy and precision. The MIP-based method, unlike the other methods required a protein precipitation step prior to extraction to eliminate the effects of co-extracted protein. The best performance was seen with the LLE method followed by SPE on the C18 phase. The MIP-based method represented no advantage over the comparator methods for this analyte. Indeed the performance of the MIP-based method was marginally worse as leaching of low level template impurities prevented detection of the target analyte at low concentrations (5 ng mL^{-1}). This relatively poorer performance was evident as worse accuracy at low concentrations with a consequent higher limit of quantification than the conventional methods.

Keywords: Solid phase extraction; Liquid–liquid extraction; Molecularly imprinted polymers; Phenyl boronic acid; Plasma; β-Blockers

1. Introduction

The potential of molecular imprinted polymers (MIPs) to act as specific solid phase extraction (SPE) sorbents for the trace analysis of compounds in a variety of matrices has been shown to be feasible by a range of studies from various groups (reviewed in [1–4]). To date the bulk of the investigations into the application of MIPs in sample preparation have focused on the feasibility of the approach [5,6] whilst few have undertaken a thorough investigation or validation of potential applications [7–9].

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Within our previous studies [10–14] we have investigated the application of MIPs in sample preparation but have ourselves not performed a rigorous comparison to conventional extraction methodologies.

As part of our own studies [15] to evaluate the use of MIPs in sample preparation we have recently investigated the extraction of M47070, an analogue of the β-blocking drug propranolol, from blood plasma onto a MIP prepared using propranol as the template. This study demonstrated that, using this extraction procedure, it was possible to develop and validate a sensitive HPLC-fluoresence-based assays for M47070. It was noteworthy that a reversed-phase (RP) method on the MIP was marginally superior to the normal phase "solvent switch" method. The limiting factor in the performance of both the reversed and normal phase MIP methods was the leaching of a template impurity from the MIP that reduced accuracy at low concentrations.

Whilst the potential use of MIPs has been demonstrated what has not been convincingly shown is that these MIP-based methodologies actually have significant advantages compared to conventional techniques. We have therefore undertaken a further investigation where the best MIP-based extraction method obtained in the aforementioned [15] study has been compared with optimised conventional methods, such as liquid–liquid (LLE) and reversed-phase SPE, in order to determine the value of MIPs for sample extraction. Within this study we have also examined how both conventional and MIP-based methods compare with a selective sample preparation approach employing reversible covalent bond formation to a phenyl boronic acid (PBA) bonded SPE phase.

2. Experimental

2.1. Materials and methods

Propranolol, M47070 and M45655 (Fig. 1) were obtained from AstraZeneca, Alderley Park, Cheshire, UK. The synthesis of the propranolol-based MIP used in this study was performed using a method based on that of Andersson [16], though with a different template:monomer:cross linker ratio (1:2:12). Propranolol, methacrylic acid (MA, monomer), ethylene glycol dimethacrylate (EGDMA, cross-linker), toluene and finally AIBN (2,2′-azobis(2-isobutyronitrile) initiator) were weighed into a glass flask and stirred on ice to dissolve the reagents. The flask was immersed in liquid nitrogen to freeze the solution and then connected to a vacuum line to evacuate the air. After returning to room temperature the flask was immersed in a glycerine bath at 60 °C to initiate the polymerisation and was maintained at this temperature overnight (16 h) until the polymerisation was complete. The MIP had template:monomer:cross linker (propranolol:methacrylic acid:ethylene glycol dimethacrylate) molar ratios of 1:2:12. The monolith polymer was crushed with a pestle and mortar and then dry sieved through a 50 μm sieve. Fines were removed by repeated sedimentation in ethanol. Following preparation, the polymers underwent extensive solvent extraction to recover template prior to use, using the solvents; ammonium acetate (1 M) dissolved in a mixture of ethanol:acetic acid:water (40:25:35; v/v/v); acetic acid:ethanol (1:3) and methanol as described in [16]. The recovered particles were dried in a vacuum oven at 50 °C and stored at ambient temperature. Investigations of the selectivity of this

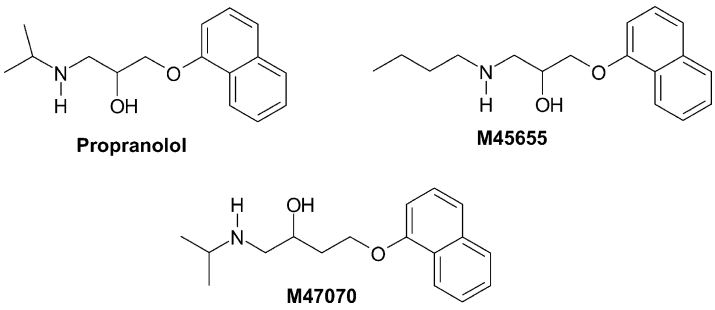

Fig. 1. Structures of propranolol, M47070 and M45655.

propranolol-imprinted MIP have been reported previously [12]. For the SPE experiments 30 mg of polymer were packed into empty SPE cartridges (Jones Chromatography Ltd, Hengoed, UK). Each cartridge was then washed again with methanol:trifluoroacetic acid (TFA) 99:1 (v/v) to minimise interference from the residual template.

2.2. Methods for the extraction of M47070 and M45655

The extraction protocols used to perform the validation experiments for each extraction method were as follows.

2.2.1. MIP-RP SPE

The extraction of plasma samples onto the MIP method was performed on human plasma samples (0.5 mL) that had been mixed with acetonitrile (1 mL) to precipitate proteins. The internal standard (M45655) was present at a concentration of 40 ng mL^{-1} in all samples. The precipitated proteins were removed by centrifugation (3000 × *g*, 5 min) prior to the application of the sample to preconditioned cartridges. The phase was conditioned by washing with methanol:TFA 99:1 (1 mL), methanol (1 mL), water (1 mL) and water:acetonitrile 1:2 (1 mL). The supernatant from the protein-precipitated sample (∼1.4 mL) was then applied, following which the cartridge was washed with water (1 mL) and water:methanol:TEA 40:60:1 (1 mL). Recovery of the extracted analytes was performed by eluting with methanol:TFA (2 × 1 mL). The eluting solvent was reduced to dryness using a stream of oxygen-free nitrogen at 30 °C and the sample re-dissolved in 250 μL of HPLC mobile phase for analysis by HPLC as described below.

2.2.2. Phenyl boronic acid-SPE

The method used to extract the analyte and internal standard was based on the methodology described in detail elsewhere [17]. Extraction on to the PBA cartridges (50 mg. 1 mL, IST, Hengoed, UK) was performed following conditioning with methanol (1 mL) and 0.1 M glycine buffer (pH 8, 2 × 1 mL). The plasma sample (0.5 mL), mixed with an equal volume of glycine buffer was then applied to the cartridge, followed by a water wash (1 mL). The cartridge was then washed with methanol water (40:60, 2 × 1 mL) and the analytes were eluted with methanol:TFA (99:1, 2 × 1 mL). The eluting solvent was reduced to dryness using a stream of oxygen free nitrogen at 30 °C, and redissolved in the HPLC mobile phase (250 μL) for analysis.

2.2.3. C18-SPE

The extraction procedure for the analytes onto C18 bonded cartridges (100 mg mL, IST, Hengoed, UK) was based on that described in detail elsewhere [18]. Prior to extraction the cartridges were conditioned sequentially with methanol (1 mL), water (1 mL) and 0.2 M sodium acetate buffer (pH 5, 1 mL). The plasma sample (0.5 mL), mixed with an equal volume of acetate buffer, was then applied to the cartridge. This was followed by washes of water (1 mL) and acetonitrile (0.5 mL) and then elution with methanol:TFA (99:1, 2 × 1 mL). The eluting solvent was reduced to dryness using a stream of oxygen free nitrogen at 30 °C, and re-dissolved in the HPLC mobile phase (250 μL) for analysis.

2.2.4. Liquid–liquid extraction

Liquid–liquid extraction was performed using an unpublished method previously validated at AstraZeneca. Plasma samples (0.5 mL) were mixed with an equal volume of 0.1% (w/v) sodium hydroxide solution and then extracted with ethyl acetate (7 mL) by vortexing for 30 s. The sample was then centrifuged for 5 min to separate the layers (1000 × *g*). Following centrifugation an aliquot (6 mL) of the organic layer was taken and reduced to dryness under a stream of oxygen-free nitrogen at 30 °C. The residue was re-dissolved in HPLC mobile phase (250 μL) for analysis.

2.3. Chromatography

The following HPLC system was used to quantify M47070 and M45655 in the MIP, C18 and PBA SPE eluents. The separation was performed on a HiChrom RPB reversed-phase HPLC column (HiChrom, Theale, UK), 15 cm × 4.6 mm i.d. 5 μm particle size, using a solvent system of methanol:water:TFA:ammonium acetate, 490:510:1:7.7 (v/v/v/w) at a flow rate of 1 ml min^{-1}. For the LLE method a solvent of methanol:water:TFA:ammonium

acetate, 600:400:1:7.7 (v/v/v/w) was used. The solvent was delivered using a LCD Analytical Constametric 3200 HPLC pump (Stone, UK) with detection using a LDC Analytical 4100 fluorescence detector (Stone, UK) operating at an excitation wavelength of 290 nm, and an emission wavelength of 340 nm. Samples were dissolved in 250 μL of mobile phase and samples (50 μL) were injected via a Perkin-Elmer ISS 200 autosampler (Beaconsfield, UK).

2.4. Validation

The aspects of method validation assessed here were extraction efficiency (recovery), linearity, accuracy, precision and matrix interference. These are in keeping with the broadly accepted principles applied to method validation [19]. The concentration ranges examined for the analyte reflect the typical clinical range that would be expected for such agents.

2.4.1. Extraction efficiency

The extraction efficiency of compound M47070 was assessed at 5 and 50 ng mL^{-1} and at 40 ng mL^{-1} for the internal standard, from spiked human plasma. Extraction efficiency was determined by comparison of peak heights of the respective compounds following extraction to those obtained for un-extracted samples.

2.4.2. Linearity

The linearity of the methods was examined by extracting a standard curve and determining the correlation coefficient, slope and intercept. The calibration line was fitted using un-weighted linear regression without forcing through the origin.

The calibration range was 0, 2, 5, 10, 20, 40 and 60 ng mL^{-1} of M47070 with samples made up in 0.5 mL of human plasma.

2.4.3. Accuracy and precision

Accuracy and precision was assessed at four concentrations within the calibration range (2, 5, 20 and 50 ng mL^{-1}) by extracting four plasma samples spiked at each concentration. Results were derived from the corresponding calibration curve.

Inaccuracy was determined from the following equation:

$$\frac{\text{concentration determined} - \text{spiked concentration}}{\text{spiked concentration}} \times 100$$

Imprecision was determined from the following equation:

$$\frac{\text{S.E.}}{\text{mean of determined concentration}} \times 100$$

where S.E. is the standard error.

2.4.4. Matrix interference

To assess the effect of interference from plasma matrix components, for each extraction method, an experiment was performed which involved extraction of plasma from three different subjects. Two samples from each subject were taken through each extraction procedure. Following extraction, one extract from each subject was spiked with M47070 and M45655 at 5 and 40 ng mL^{-1}, respectively. In addition, un-extracted samples were prepared (at the same concentrations as the extracted samples) directly into clean tubes. Samples were re-dissolved in mobile phase (250 μL) and 50 μL were injected.

3. Results and discussion

3.1. Extraction efficiencies

All the extraction methods employed were found to have high extraction yields at both 5 and 50 ng mL^{-1}. These data are summarised in Table 1 and show that the highest extraction efficiencies were seen for liquid–liquid extraction. However, LLE showed relatively poor reproducibility at 5 ng mL^{-1} and in addition, the variability seen in extraction efficiency for the internal standard was also high. Extraction onto C18 bonded silica gel was relatively constant at

Table 1
Extraction efficiencies (±S.D.) for all extraction methods assessed

Method	5 ng mL^{-1} M47070	50 ng mL^{-1} M47070	Internal standard
MIP (RP)	87.4 (2.02)	81.2 (2.71)	84.1 (3.85)
LLE	109.8 (15.6)	96.4 (0.89)	101.0 (21.8)
C18-SPE	89.8 (1.95)	90.8 (2.46)	90.3 (2.10)
PBA-SPE	96.2 (5.10)	73.4 (4.20)	96.9 (5.05)

Table 2
Characteristics of calibration curves obtained using each method

Method	Slope	Intercept	R^2
MIP	0.0249	+0.0373	0.9984
LLE	0.0296	+0.0109	1.0000
C18-SPE	0.0281	−0.00005	0.9998
PBA-SPE	0.0267	−0.0006	0.9987

ca. 90% for M47070 across the concentration range examined, and showed excellent reproducibility of extraction at both high and low concentrations. With both the MIP and PBA-based methods a decline in extraction efficiency was noted at 50 ng mL^{-1} compared to 5 ng mL^{-1} (particularly noticeable for the PBA phase) suggesting saturation of the extraction mechanism. The MIP-based method gave very reproducible extraction efficiencies at both concentrations, whilst the results for PBA, although acceptable, showed more variability.

3.2. Linearity

On the basis of the studies undertaken here all of the extraction methods resulted in linear calibration curves over the range of concentrations examined (0–60 ng mL^{-1}).

Details of the slope, intercept and R^2 values for each method are provided in Table 2. The small differences in the correlation coefficient between assay methods were not believed to represent significant differences but more probably the variability associated with manual spiking methods. It was noticeable that the MIP-based method had a higher intercept than the other methods that was attributed to interference of the M47070 peak in this method leading to slight overestimation of peak height.

3.3. Accuracy and precision

Arguably the characteristics that most define the performance of an analytical method are accuracy and precision. The results for accuracy and precision, estimated over the range 2 to 50 ng mL^{-1}, are given in Table 3. Perhaps surprisingly, given their unfashionable status, the most accurate and precise method was found to be LLE. This was followed by solid-phase extraction on to C18 bonded silica gel, which also gave a high degree of precision, but slightly overestimated concentrations at 2 and 5 ng mL^{-1} (although these were well within the generally accepted criteria for method validation). For both of these extraction techniques the methods would have been suitable for the analysis of plasma samples with limits of quantification of 2 ng mL^{-1}. As reported previously [15] the precision of the MIP-based method was acceptable over the whole range, though less good than either liquid–liquid or C18 SPE. However, at the lowest concentration examined accuracy was poor. In the case of the PBA-based solid phase extraction method precision was acceptable over the whole range of concentrations examined but accuracy was generally poor. Thus, at the low concentrations (2 and 5 ng mL^{-1}) the results overestimated the amount of M47070 present, whilst at the top concentration the quantities of the analyte were underestimated.

Table 3
Accuracy and precision data for each of the assays

Method	2 ng mL^{-1}	5 ng mL^{-1}	20 ng mL^{-1}	50 ng mL^{-1}
MIP				
Accuracy	158.0	115.0	99.5	101.4
Precision	10.24	4.60	2.30	1.59
LLE				
Accuracy	101.3	101.6	100.7	98.1
Precision	1.92	2.06	0.67	0.89
C18-SPE				
Accuracy	109.3	104.6	100.3	96.1
Precision	1.41	0.39	1.86	1.17
PBA-SPE				
Accuracy	125.2	123.9	95.7	88.5
Precision	2.47	3.73	1.91	1.77

3.4. Interference

One of the factors that limit the performance of many analytical methods is the presence of interferences that are derived either from the matrix or result from the methodology used to prepare the sample. Indeed, one of the major reasons for sample preparation is the removal of such interferences. Whilst the analytical figures of merit, such as accuracy and precision, give a good indication of how well these interferences have been eliminated, examination of the chromatograms for the extracts obtained by the various methods provides a second, qualitative, estimate

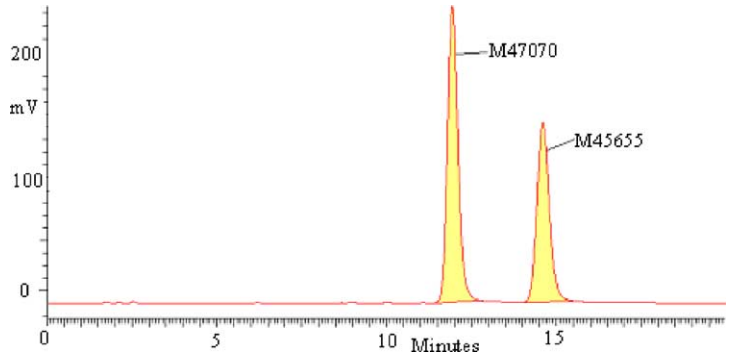

Fig. 2. Chromatogram of an un-extracted standard of M47070 at 60 ng mL^{-1} and M45655 at 40 ng mL^{-1}.

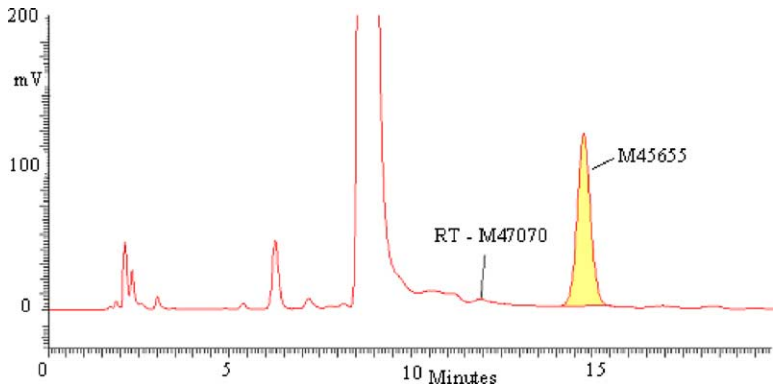

Fig. 3. Chromatogram of blank standard (including internal standard) for MIP-RP—propranolol elutes at 9 min.

of the efficiency of the sample preparation step. In our previous study, where the MIP-based extraction was optimised, it became clear that whilst this approach removed the bulk of the endogenous interferences, template-derived material that co-chromatographed with the analyte was present in the extracts.

Example HPLC-fluorescence chromatograms from blank plasma samples (including internal standard) from each of the assays are presented in Figs. 2–6. As can be seen from these figures the baseline obtained for the MIP-based extraction showed very few interferences in the region of the solvent front. The large peak

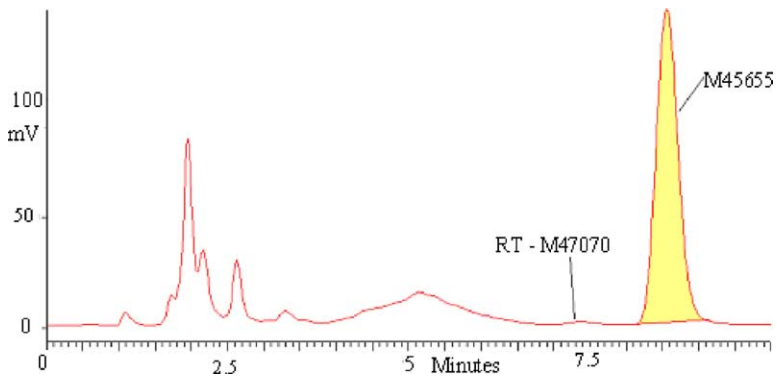

Fig. 4. Chromatogram of blank standard (including internal standard) for LLE.

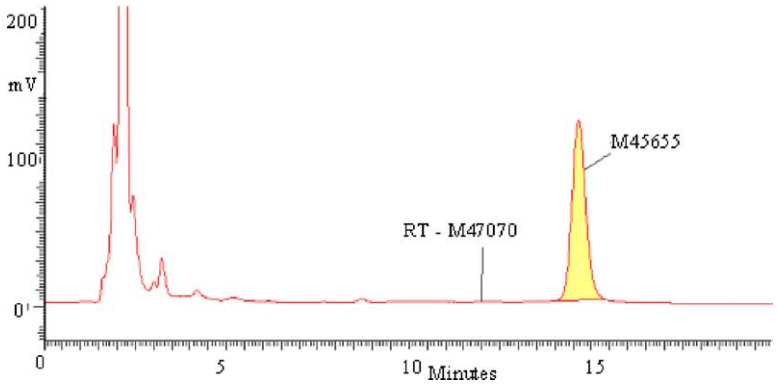

Fig. 5. Chromatogram of blank standard (including internal standard) for C18.

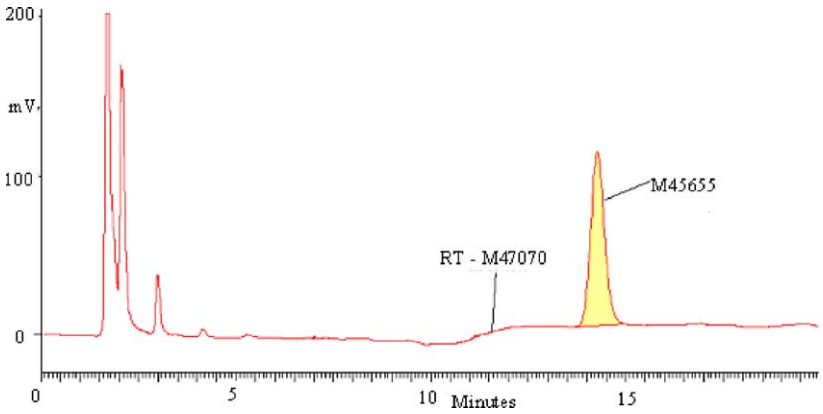

Fig. 6. Chromatogram of blank standard (including internal standard) for PBA.

present at approximately 9 min was due to leaching template which was still present in the polymer despite exhaustive washing prior to use. The good separation of the template peak from M47070 and M45655 meant that quantification was not compromised by the presence of propranolol in the eluates. However, examination of the chromatogram at the retention time of M47070 revealed the presence of minor components due to the template (they were not seen with the blank polymer [15]). These were possibly either impurities of the bulk template or breakdown products produced during polymerisation. The presence of these components affected accuracy and precision at low concentrations of M47070.

In the case of the PBA, C18 and solvent extractions, the solvent front region of the chromatograms contained more co-extracted peaks than were observed with the MIP. Within this group the C18-based extracts contained more co-extracted material than either the PBA or solvent extraction methods. However, despite the evidence of co-extracted material in the region of the solvent front, the remainder of the chromatograms for all three methods were free of interferences. As shown in Figs. 4–6, this was particularly the case around the retention times for M47070 and M45655 and none of these methods had peaks for co-extracted substances which would have compromised the detection of the analytes.

4. Discussion

The aim of this investigation was to determine how well an optimised MIPSPE method compared to more

conventional sample preparation techniques. With respect to removal of endogenous interferences it is arguable that the MIP-based procedure was indeed more effective than the other methods, but this advantage was then lost because of the template-related interferences. Clearly, the only relevant measure of sample clean up is the presence or absence of interfering peaks in the region of interest, and by this criterion extraction on to this MIP produced less impressive results compared to the other methods. In addition the presence of the residual template required a much longer HPLC run time than would have been required had it been possible to remove it from the MIP prior to analysis. The MIP method, unlike the other extraction techniques investigated, also required the removal of plasma proteins via precipitation with acetonitrile, and centrifugation prior to extraction [15]. Thus, the overall conclusion from this series of experiments was that, whilst it was possible to generate a validated MIP-based sample preparation procedure for the analyte spiked into plasma, there appeared to be no real advantage to this over conventional extraction methods. Indeed, the conventional methods of LLE and SPE on C18 bonded silica gel gave superior results to both of the "specific" methods based on either molecular imprinting or covalent bond formation.

These conclusions must clearly be qualified to indicate that they relate to the investigation of one MIP, two specific compounds and detection by fluorescence and thus cannot necessarily be extrapolated to other compounds. Fluorescence detection is inherently more selective than UV and may well have shown up all methods as producing clean extracts whereas UV detection, particularly at low wavelength 210–220 nM would have provided a fuller indication as to the cleanliness of extracts. However, fluorescence detection was needed in this assay to obtain the required sensitivity of detection for the analyte and internal standard. In the recent example provided by Andersson et al. [9], where GC and HPLC–MSMS have been applied to the analysis of local anaesthetics, the MIP provided excellent results in comparison to LLE and SPE on a C18 bonded phase. Our results do, however, illustrate the potential problems that arise for MIP-based methods based on the simple "first generation" of MIPs where leaching is evident. It is perhaps noteworthy that leaching was not particularly evident in the study by Andersson et al. [9] mentioned above. However, work by Blomgren et al. [20] on the HPLC–UV analysis of clenbuterol in calf urine showed that whilst acceptable accuracy and precision were obtainable using a MIP (prepared from brombuterol) leaching of the template was still significant (though, in this instance, not a cause of interference). Thus leaching of the template is therefore probably the main problem to be solved before MIPs will offer a real challenge to conventional methods.

The work of Sanbe [21] illustrates another approach to the extraction of β-blockers. This approach involved a restricted access media approach using propranolol as the template. Based on our results, where a prior protein precipitation step was necessary, it appears that the use of restricted access media could reduce the extent of protein co-extraction and this, in combination with MIPSPE, could well represent a way forward.

Although use of a structural analogue as template (dummy template approach) as illustrated here, overcomes some leaching problems (see also [20]), any template-derived impurities (even at low levels) can still confound successful method development. It could of course be argued that if HPLC with mass spectrometry (MS) had been used rather than HPLC-fluoresence the interference from the template-derived impurity would have been eliminated. However, whilst this is almost certainly the case, the use of HPLC–MS would in all probability also have removed the need for the selectivity in sample preparation that is suggested as the major reason for employing MIPs in the first place.

With HPLC–MS becoming the method of choice (at least within the pharmaceutical industry) for the determination of drugs in biological fluids at trace concentration it will be interesting to see whether the combination of a MIP-extraction with HPLC–MS will prove to have advantages over conventional extraction prior to HPLC–MS. The logic being that a more selective extraction could lead to less ion suppression in the MS and more accurate and precise results. Study is required in this area, and we hope to investigate this in due course.

References

[1] J. Olsen, P. Martin, I.D. Wilson, Anal. Commun. 35 (1998) 13H–14H.

[2] D. Stevenson, Trends Anal. Chem. 18 (1999) 154–158.
[3] L.I. Andersson, J. Chromatogr. B 739 (2000) 163–173.
[4] L.I. Andersson, L. Scheitz, in: I.D. Wilson (Ed.), Handbook of Analytical Separations: Bioanalytical Separations, vol. 4, Elsevier, 2003, pp. 45–71.
[5] R.F. Venn, R.J. Goody, in: E. Reid, H.H. Hill, I.D. Wilson (Eds.), Methodological Surveys in Bioanalysis of Drugs, vol. 25, Royal Society of Chemistry, Cambridge, 1998, pp. 13–27.
[6] R.F. Venn, R.J. Goody, Chromatographia 50 (1999) 407–412.
[7] L.I. Andersson, A. Paprica, T. Arvidsson, Chromatographia 46 (1997) 57–62.
[8] L.I. Andersson, Analyst 125 (2000) 1515–1517.
[9] L.I. Andersson, M. Abdel-Rehim, L. Nicklasson, L. Schweitz, S. Nilsson, Chromatogr. Suppl. 55 (2002) S-65–S-69.
[10] P. Martin, I.D. Wilson, G.R. Jones, K. Jones, in: E. Reid, H.H. Hill, I.D. Wilson (Eds.) Methodological Surveys in Bioanalysis of Drugs, vol. 25, Royal Society of Chemistry, Cambridge, 1998, pp. 21–27.
[11] P. Martin, I.D. Wilson, E.D. Morgan, G.R. Jones, K. Jones, Anal. Commun. 34 (1997) 45–47.
[12] P. Martin, I.D. Wilson, G.R. Jones, J. Chromatogr. A 889 (2000) 143–147.
[13] J. Olsen, P. Martin, I.D. Wilson, G.R. Jones, Analyst 124 (1999) 467–471.
[14] P. Martin, I.D. Wilson, G.R. Jones, Chromatogr. Suppl. 52 (2000) S-19–S-23.
[15] P.D. Martin, G.R. Jones, F. Stringer, I.D. Wilson, Analyst 128 (2003) 345–350.
[16] L.I. Andersson, Anal. Chem. 68 (1996) 111–117.
[17] P. Martin, B. Leadbetter, I.D. Wilson, J. Pharm. Biomed. Anal. 11 (1993) 307–312.
[18] P. Martin, J. Taberner, A. Fairbrother, I.D. Wilson, J. Pharm. Biomed. Anal. 11 (1993) 671–677.
[19] V.P. Midha, K.K. Shah, S. Dighe, I.J. McGilevary, J.P. Skelly, A. Yacobi, T. Layoff, C.T. Viswanathan, C.E. Cook, R.D. McDowall, K.A. Pittman, S. Spector, J. Pharm. Sci. 81 (1992) 309–312.
[20] A. Blomgren, C. Berggren, A. Holmberg, F. Larsson, B. Sellergren, K. Ensing, J. Chromatogr. A 975 (2002) 157–164.
[21] H. Sanbe, J. Haginaka, Analyst 128 (2003) 593–597.