Journal of Pharmaceutical & Biomedical Analysis
Vol. 11, No. 4/5, pp. 307–312, 1993
Printed in Great Britain

0731–7085/93 $6.00 + 0.00

Immobilized phenylboronic acids for the selective extraction of β-blocking drugs from aqueous solution and plasma

P. MARTIN,* B. LEADBETTER and I.D. WILSON

Department of Safety of Medicines, ICI Pharmaceuticals, Mereside, Alderley Park, Macclesfield SK10 4TG, UK

Abstract: The use of phenylboronic acid (PBA) solid-phase extraction cartridges for the extraction of a range of β-blockers from aqueous buffer and plasma has been investigated and compared with other phases commonly used for solid-phase extraction. PBA was found to provide an efficient means for the extraction of this class of compound from simple aqueous buffer systems. Extraction from buffers was pH-dependent and gave optimum results at approximately pH 8. Extraction from plasma was less efficient for some of the test compounds; this matrix effect was probably due to protein binding. Chromatography showed that plasma extracts were free of major sources of interference.

Keywords: *Phenylboronic acid; PBA; solid-phase extraction; SPE; β-blockers.*

Introduction

The β-blockers form a large and important group of drugs. Earlier work in this department has been concerned with the development of solid-phase extraction (SPE) methods for use in the preparation of samples for the analysis of this class of compounds [1–3]. The wide range of phases available for use in SPE provides a variety of extraction mechanisms. These include essentially non-polar interactions (e.g. C18, C8, C2, phenyl, cyclohexyl and cyanopropyl-bonded silica gel or materials such as graphitized carbon [1]), polar interactions (e.g. silica gel, diol, cyanopropyl) and either weak or strong ion exchange (e.g. carboxylic acid, aminopropyl, strong anion- or strong cation-exchange phases). Some phases can exhibit more than one mechanism simultaneously thus providing a 'mixed mode' of extraction. Examples include the extraction of β-blockers on to C18 bonded silica gel by both hydrophobic and ion-exchange mechanisms, the latter resulting from the presence of residual silanols [2, 3] or the deliberate preparation of multifunctional phases (e.g. the 'Certify' range from Varian). A potentially more specific extraction mechanism, suitable for a relatively restricted range of compounds (e.g. refs 4–9), involves covalent bond formation with immobilized phenylboronic acid (PBA). The presence of a hydroxypropylamino function in β-blockers provides a suitable group for the formation of cyclic boronates and indeed has been used to provide volatile derivatives for their analysis by gas chromatography [10]. In the present paper some preliminary investigations on the use of PBA bonded to silica gel are described for the extraction of a range of β-blockers from aqueous buffers and plasma.

Materials and Methods

Chemicals

The [^{14}C]-labelled compounds used in this study were: practolol, (2*RS*)-3-(4-acetamidophenoxy)-1-isopropylamino-2-propanol (8.8 μCi mg^{-1}); propranolol, (2*RS*)-1-isopropylamino-3-(1-naphthyloxy)-2-propanol (38.4 μCi mg^{-1}); ICI 118,551, (2*RS*,3*RS*)-1-(7-methylindan-4-yloxy)-3-isopropyl-aminobutan-2-ol (10.6 μCi mg^{-1}); and epanolol, *N*-(2-[(2*RS*-3-*o*-cyanophenoxy-2-hydroxypropyl]-aminoethyl)-4-hydroxyphenyl-acetamide (13.1 μCi mg^{-1}) (Fig. 1, 1–4); these compounds were synthesized in the radiochemical laboratory, ICI Pharmaceuticals. The radiochemical purity of the compounds, determined by thin-layer chromatography, was greater than 95%.

* Author to whom correspondence should be addressed.

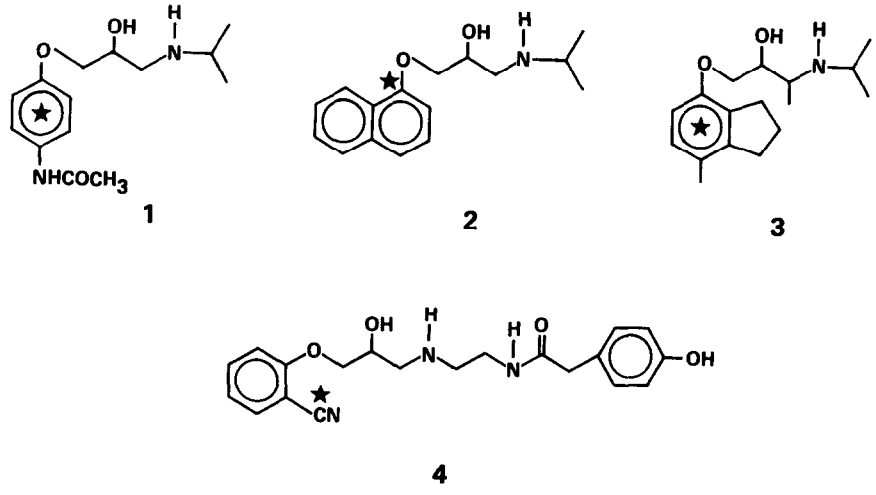

Figure 1
Structures of test compounds. 1, practolol; 2, propranolol; 3, ICI 118,551; 4, epanolol.

All solvents and chemicals were obtained from E. Merck (Poole, UK) or Fisons (Loughborough, UK) and were of Analar or HPLC grade.

SPE cartridges

100-mg (1-ml) C18, phenyl, propylamino, silica gel and PBA Bond Elut SPE cartridges (Varian Assoc.) were obtained from Jones Chromatography Ltd (Hengoed, UK).

Solid-phase extraction

Aqueous buffer. The extraction protocol used for the bulk of the studies described here was optimized for the extraction of propranolol. Samples, comprising the [^{14}C]-labelled analyte spiked into glycine buffer (0.1 M, pH 8.2) at a concentration of 10 μg ml^{-1}, were applied to 100 mg (1 ml) PBA columns conditioned with 1 ml of methanol and 5 ml of glycine buffer. After application of the drug, the cartridges were washed with 1 ml of deionized water and then with 3 ml of methanol–water (40:60, v/v) to remove non-specifically retained buffer components. Finally the retained β-blockers in the cartridges were eluted with 3 ml of water–methanol–trifluoroacetic acid (TFA) (50:50:1, v/v/v).

The eluates were collected and analysed by scintillation counting. For analysis 10 ml of Beckman Ready Value scintillant was added and the radioactivity was determined in a Beckman model LS5801 scintillation counter.

The same sample application and elution conditions were also used for the other SPE cartridges examined in order to be able to make direct comparisons between the various phases.

The influence of the pH of the sample at the application step was investigated for PBA by applying samples in glycine buffer at pH 2, 4, 6, 8, 10 or 12. The conditioning wash and elution steps were unchanged.

Rat plasma. Each of the test compounds dissolved in methanol (1 mg ml^{-1}) was spiked into 0.5 ml of rat plasma at a final concentration of 10 μg ml^{-1}. The sample was then mixed with 0.5 ml of glycine buffer (pH 8.2) and applied to preconditioned PBA cartridges. For some compounds further experiments were undertaken using increasing proportions of glycine buffer. To investigate the effects of matrix components on extraction, plasma filtrates were prepared by centrifuging (3000 rpm for 10 min) control rat plasma through a Sartorious Centrisort 1 (SM 132 39E) membrane with a 10,000 mw cut-off.

Chromatographic analysis of propranolol extracts from human plasma. Control human plasma was spiked with [^{14}C]-propranolol at a final concentration of 1 μg ml^{-1} and extracted on to the PBA phase as described above. Aliquots (10 μl) of the methanol–water–TFA eluate were then analysed by HPLC on a 150 × 4.6 mm i.d. silica gel column (5 μm Spherisorb) using a mobile phase of methanol–aqueous ammonium acetate (98:2, v/v) at a flow rate of 1 ml min^{-1} and ambient tempera-

ture. Detection was by UV absorbance at 296 nm. This procedure was also applied to unspiked control plasma.

Results and Discussion

Extraction of β-blockers from aqueous buffer

The extraction procedure developed for [^{14}C]-propranolol from glycine buffer involved the application of the sample at pH 8.2 to preconditioned PBA cartridges, followed by washes with water and methanol–water (40:60, v/v) and elution with methanol–water–TFA (50:50:1, v/v/v). Under these conditions highly reproducible extractions were obtained, with the bulk of the compound recovered in the final elution step (90.3 ± 1.6%) (Table 1). In order to show that this was a specific interaction with the PBA, and not a secondary interaction (e.g. with silanols) of the type observed elsewhere [2, 3], the extraction behaviour of propranolol was examined on a range of other SPE phases. Thus, the phenyl phase was investigated because the presence of a phenyl group in PBA leads to the possibility of pi–pi interactions with the aromatic ring of the analyte. Similarly the propylamino phase was studied because of the linkage of the PBA moiety via a propylamino side chain to the silica. Silica itself was studied because of the possible role of surface silanols alluded to above. The C18 phase was included for comparison with PBA because of the widespread use of this reversed-phase material in applications of this nature. The results for these experiments, where the conditioning, wash and elution steps for each of the phases were the same as those used for extraction on to PBA, are shown in Table 1. The extraction/elution profiles for the silica, aminopropyl and phenyl phases are very different from that of the PBA material, supporting the contention that a different extraction mechanism operates for the latter. For example, although silica gel gave a good initial extraction the bulk of the radiolabel was recovered in the subsequent wash steps. A similar profile was obtained for the aminopropyl material albeit with a lower initial extraction from the buffer. For the phenyl phase under these conditions the initial extraction was good but the overall recovery was low (7.4 ± 0.3%). Complete recovery from the phenyl SPE cartridges was only achieved by elution with 100% methanol. Perhaps surprisingly the phase showing the greatest similarity to the PBA under this set of extraction conditions was the C18 bonded material. However, by modifying the wash steps to include two 1 ml applications of methanol, rather than methanol–water (60:40, v/v), about 60% of the applied propranolol was eluted from the C18 phase compared to less than 15% for PBA. This result also implies an underlying difference in extraction mechanism between the C18 and PBA phases.

Whereas these experiments do not eliminate the possibility of some contribution by 'secondary' interactions, the results suggest nevertheless that it is the boronic acid group which is largely responsible for the extraction of propranolol on to the PBA phase.

The capacity of the PBA phase for the extraction of propranolol from aqueous buffer was then investigated. No difference in extraction efficiency or recovery was detected over the range 10 ng–10 μg following extraction on to 100-mg PBA cartridges (Table 2).

Under the conditions optimized for propranolol, investigations were then conducted on the extraction of practolol, epanolol and ICI 118,551 from aqueous buffer on to PBA. The results of these experiments are shown in Table 3. Good extraction efficiencies and recoveries were obtained for ICI 118,551, which indeed showed very similar behaviour to propranolol. For both practolol and epanolol good extraction from the buffer was observed but significant losses were seen during the wash steps. In the case of epanolol some material

Table 1
Comparison of PBA with other SPE phases for the extraction of [^{14}C]-propranolol*

Phase	C18	Silica	Amino	Phenyl	PBA
Sample application	2.1 ± 0.1	4.3 ± 0.1	43.0 ± 2.3	2.6 ± 0.0	2.7 ± 0.1
Wash steps	4.8 ± 0.2	85.1 ± 5.1	59.0 ± 2.0	4.0 ± 0.1	2.9 ± 0.2
Elution stop	82.2 ± 5.9	9.2 ± 1.4	3.0 ± 0.2	0.8 ± 0.2	90.3 ± 1.6
Total	89.1 ± 5.7	98.6 ± 3.8	105.0 ± 0.1	7.4 ± 0.3	95.9 ± 1.9

*The results are shown as mean ±SE (n = 3) for radioactivity in the column eluates at each step in the extraction.

also appears to have been retained on the column. The reasons for the observed differences in extraction and elution behaviour may be partly due to differences in pK_a and log P values of the various analytes. Both propranolol and ICI 118,551 have similar log P values (3.56 and 3.82, respectively) and similar pK_a values (9.45 and 9.56). In contrast both practolol and epanolol have log P values of less than 1 (0.79 and 0.87). The pK_a of practolol is 9.50 and is therefore similar to that of propranolol and ICI 118,551 but that of epanolol is only 7.72. The observed differences in recoveries may simply result from differences in polarity and ionization at pH 8.2 (the pH of the buffer). Modification of the wash steps by using lower proportions of methanol enabled losses of epanolol and practolol at this step to be minimized.

The best extraction of these β-blockers would be expected to occur under alkaline conditions where the reactive boronate form was present. In Fig. 2 the pH-extraction profile for all four test compounds is shown over the range 2–12. As is clearly apparent the optimum pH for extraction is approximately pH 8, with poor extractions below this pH and a general fall in extraction efficiency above pH 10.

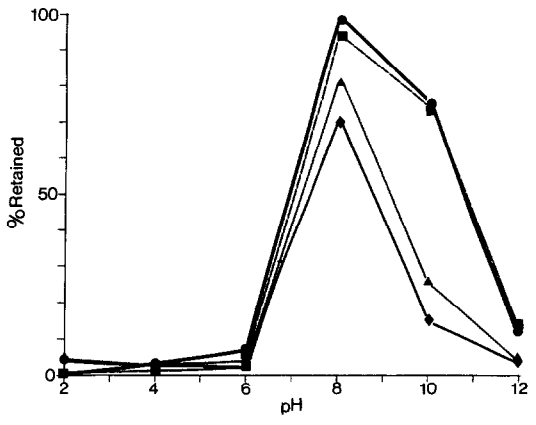

Figure 2
The pH extraction profiles for [^{14}C]-practolol, ▲; propranolol, ■; ICI 118,551, ●; and epanolol, ◆.

Table 2
Capacity of PBA columns for the extraction of [^{14}C]-propranolol from aqueous buffer*

Propranolol conc. (ng ml^{-1})	Sample application	Wash steps	Elution step
10	3.3 ± 0.2	2.2 ± 2.2	91.7 ± 0.1
100	3.1 ± 0.0	3.1 ± 0.3	93.4 ± 3.0
500	3.0 ± 0.1	3.4 ± 0.6	90.8 ± 2.1
1000	2.9 ± 0.1	3.2 ± 0.3	90.7 ± 0.2
10,000	2.7 ± 0.2	3.1 ± 0.1	92.6 ± 0.4

*The results are shown as mean ±SE (n = 3) for radioactivity in the column eluates at each step in the extraction.

Table 3
Extraction of β-blockers from glycine buffer on to PBA SPE cartridges*

Compound	Propranolol	Practolol	ICI 118,551	Epanolol
Sample application	2.7 ± 0.1	7.3 ± 0.0	0.3 ± 0.3	0.9 ± 0.1
Wash steps	2.9 ± 0.2	8.8 ± 0.2	0.8 ± 0.4	16.5 ± 0.7
Elution step	90.3 ± 1.9	82.3 ± 0.8	96.6 ± 2.5	66.2 ± 0.4
Total recovery	96.1 ± 1.9	98.4 ± 0.6	97.7 ± 2.6	83.5 ± 0.6

*The results are shown as mean ±SE (n = 10 for propranolol, n = 3 for the remaining compounds) for radioactivity in the column eluates at each step in the extraction.

Table 4
Extraction of β-blockers from rat plasma on to PBA SPE cartridges*

Compound	Propranolol	Practolol	ICI 118,551	Epanolol
Sample application	5.0 ± 0.7	8.6 ± 0.8	1.4 ± 0.1	19.5 ± 2.7
Wash steps	2.0 ± 0.1	14.4 ± 0.5	1.1 ± 0.1	25.1 ± 0.2
Elution step	88.7 ± 0.6	73.0 ± 1.1	93.6 ± 0.4	41.8 ± 2.4
Total recovery	95.7 ± 0.0	96.0 ± 0.8	96.2 ± 0.4	86.4 ± 1.1

*The results are shown as mean ±SE (n = 3) for radioactivity in the column eluates at each step in the extraction.

Extraction of β-blockers from rat and human plasma

After it had been established that the PBA phase was capable of extracting β-blockers from aqueous buffer solutions, examination of this phase was extended to animal and human plasma. The conditions employed were those developed for propranolol. Plasma spiked with the test analyte was mixed with an equal volume of glycine buffer and applied to preconditioned cartridges; the results are shown in Table 4. [^{14}C]-propranolol showed a similar extraction profile from both plasma and aqueous buffer. A typical chromatogram showing the results of the HPLC analysis of a human plasma extract spiked with 1 μg of propranolol (with extraction efficiency similar to that observed for rat plasma) is shown in Fig. 3(a). This chromatogram was obtained by the injection of approximately 50 ng of propranolol on the column. Comparison of this chromatogram with that obtained for a control plasma extract examined by the same procedure [Fig. 3(b)] shows the absence of interfering peaks in the control plasma extract. Whereas the results for the extraction of propranolol and ICI 118,551 from plasma were very similar to those obtained for these drugs from buffer, a clear matrix effect was observed for the remaining two compounds. This was shown by greater losses in both the application and wash steps. For example the losses of epanolol on application in plasma were about 20% compared with less than 1% from aqueous buffer. An obvious explanation for such an effect is the possibility of plasma protein binding of the analyte.

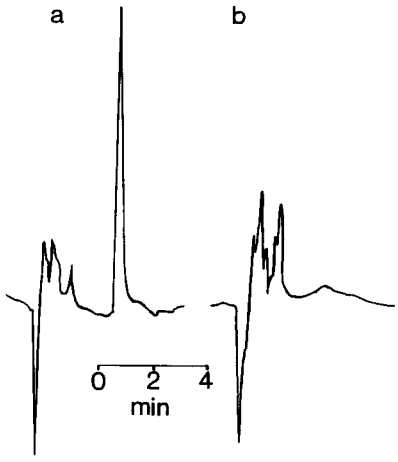

Figure 3
Chromatograms obtained following the extraction of (a) propranolol from spiked human plasma, and (b) control human plasma on phenylboronic acid SPE cartridges.

Therefore samples were applied to PBA cartridges after mixing with increasing proportions of glycine buffer; the results are shown in Fig. 4. For propranolol, ICI 118,551 and practolol dilution of the sample had relatively little effect on extraction; this result was not surprising in the case of propranolol and ICI 118,551 as there was no evidence for a matrix effect in the original extraction. In contrast dilution resulted in a large increase in the amount of epanolol extracted.

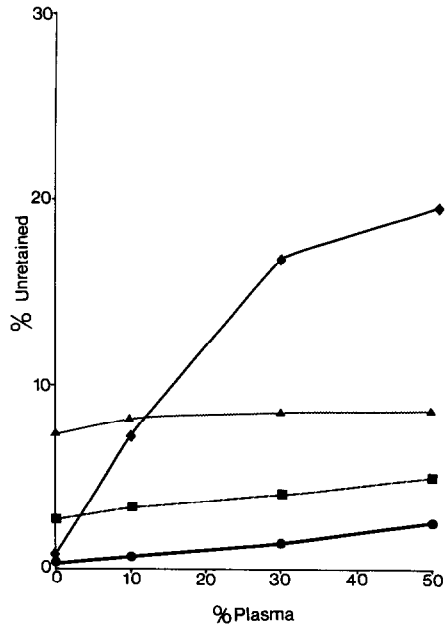

Figure 4
Effect of dilution of rat plasma samples with glycine buffer on the extraction of [^{14}C]-β-blockers on to phenylboronic acid SPE cartridges. Key as for Fig. 2.

This phenomenon was further investigated by extracting epanolol from a plasma ultrafiltrate, obtained using ultrafiltration with a 10,000 mw cut-off. Since only 3% of this sample was lost in the application step, plasma protein binding is clearly implicated as the cause of the poor extraction of this compound from whole plasma.

Conclusions

The PBA phase provides an interesting alternative to the more commonly used SPE materials for the extraction of β-blockers. The results presented are consistent with the formation of cyclic boronate esters and may

therefore provide the basis for highly selective analytical methods. Further studies are continuing to investigate the usefulness of immobilized PBA and to fully define the physicochemical parameters which are important for the extraction of β-blockers on to this phase.

References

[1] R. Roberts, R.J. Ruane and I.D. Wilson, *J. Pharm. Biomed. Anal.* **7**, 1077–1086 (1989).
[2] R.J. Ruane and I.D. Wilson, *J. Pharm. Biomed. Anal.* **5**, 723–727 (1987).
[3] R.J. Ruane, I.D. Wilson and G.P. Tomkinson in *Bioanalysis of Drugs and Metabolites* (E. Reid, J.D. Robinson and I.D. Wilson, Eds), pp. 295–298. Plenum, New York (1988).
[4] R.R. Maestas, J.R. Priesto, G.D. Kuehn and J.H. Hageman, *J. Chromatogr.* **189**, 225–231 (1980).
[5] F.H. Pfadenhauer and S. Tong, *J. Chromatogr.* **162**, 585–590 (1979).
[6] A.H. Wu and T.G. Garnet, *Clin. Chem.* **31**, 298–302 (1985).
[7] M.L. Stolwitz, in *Proceedings of the Second International Symposium on Sample Preparation*, pp. 41–44. Analytichem International, CA, USA (1985).
[8] M. Tugnait, F.Y.K. Ghauri, I.D. Wilson and J.K. Nicholson, *J. Pharm. Biomed. Anal.* **9**, 895–899 (1991).
[9] I.D. Wilson, E.D. Morgan and S.J. Murphy, *Analytica Chimica Acta* **236**, 145–155 (1990).
[10] C.F. Poole and S.A. Schuette, *Contemporary Practice of Chromatography*, p. 507. Elsevier, Amsterdam (1984).

[Received for review 11 May 1992; revised manuscript received 24 July 1992]