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An investigation of the effects of carbon loading and endcapping on the solid-phase extraction of β-blockers onto C_{18} bonded silica gel

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Abstract: The effects of carbon loading and endcapping on the solid-phase extraction onto C_{18} bonded silica gel of a range of β-blockers from aqueous buffer and from dog plasma has been investigated. The highest extraction efficiencies were obtained for those phases with carbon loadings of between 5 and 16% for phases without endcapping or 10.5–14% for endcapped material. With carbon loadings of 18 and 22% (plus endcapping) poor extractions from the matrix were obtained combined with further losses at the wash steps. Matrix effects were observed with dog plasma which accentuated the effects seen with buffer. These results are best explained by assuming that a cationic interaction of the secondary amino group present in the analytes with residual silanols on the silica surface is primarily responsible for the extraction of these analytes.

Keywords: *Solid-phase extraction; β-blockers; silanols; silanol interactions; carbon loading; endcapping.*

Introduction

The β-blockers form a large class of basic drugs used primarily for the treatment of heart conditions. Sensitive and specific assays for these compounds in biological fluids are a key requirement to support drug development and for subsequent therapeutic monitoring. In recent years sample preparation using solid-phase extraction (SPE) (or liquid–solid extraction, LSE) has become an increasingly popular means of obtaining samples in a suitable form for chromatographic analysis. The SPE of the β-blockers has been demonstrated on a wide range of phases including C_{18}, C_2, CN, graphitized carbon and phenylboronic acid [e.g. 1–2]. The extraction of these compounds onto the silica based 'reversed-phase' SPE materials (e.g. C_{18}) is however, complex and would appear to result from a mixture of at least two mechanisms. Thus in addition to the expected hydrophobic interaction with the phase there also appears to be a cation exchange interaction between the secondary amino group and residual silanols on the silica gel [1, 3–5] (recent studies from these laboratories have also suggested that hydrogen bonding may also contribute [6]). Clearly, in order to be able to develop reliable SPE methodologies for the extraction of basic compounds such as the β-blockers a better understanding of the relative importance of these various mechanisms in the extraction process would be of value. In the studies described here we have taken the opportunity afforded by the availability of a range of C_{18} bonded cartridges with different carbon loadings and degrees of endcapping to further investigate the contribution of this cation exchange component to the SPE of a range of β-blockers.

Experimental

Materials

A total of four [14C]-radiolabelled β-blockers (synthesized in the Radiochemical Laboratory at ICI Pharmaceuticals, Alderley Park), with a radiochemical purity of >95% were used in these studies. The compounds were practolol ((2*RS*)-3-(4-acetamidophenoxy)-1-isopropylamino-2-propanol) specific activity 8.8 μCi mg^{-1}; propranolol ((2*RS*)-1-isopropylamino-3-(1-naphthyloxy)-2-propanol), specific activity 38.4 μCi mg^{-1}; ICI 118, 551 ((2*RS*,3*RS*)-1-(7-methyl-indan-4-yloxy)-3-isopropylamino-butan-2-ol) specific activity 10.6 μCi mg^{-1}, and epanolol (N-{2-[(2*RS*)-3-*o*-cyanophenoxy-2-hydroxypropyl]-

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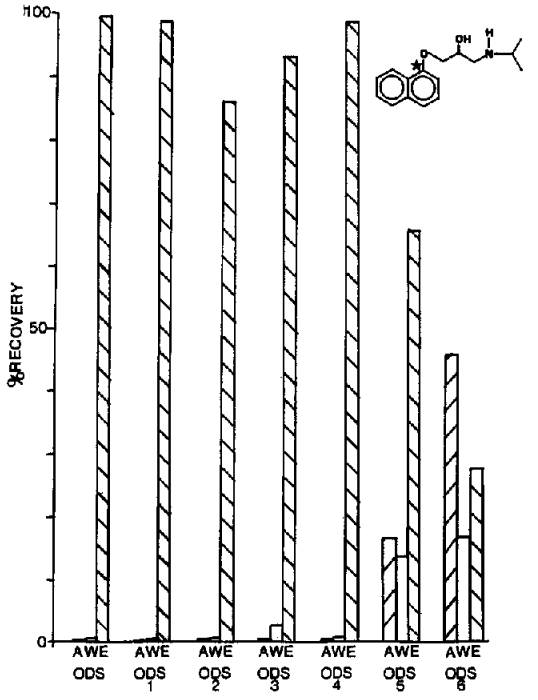

Figure 1
Recovery profile for [^{14}C] propranolol following application (A), wash (W) and elution (E) steps from Whatman SPE cartridges. Samples were applied in aqueous buffer.

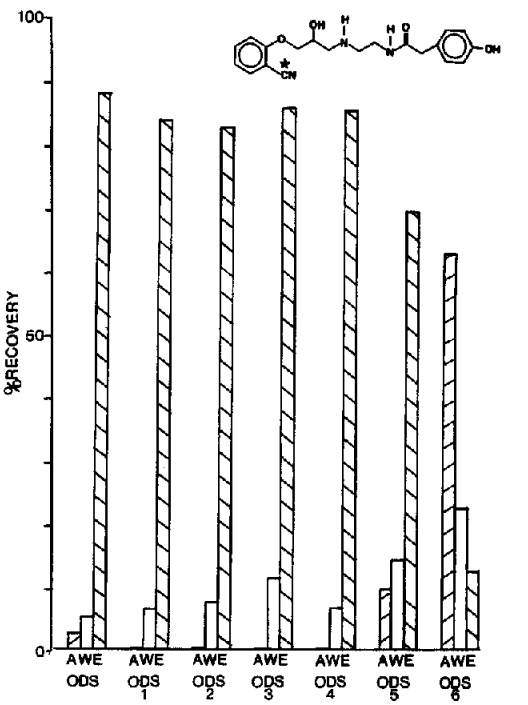

Figure 3
Recovery profile for [^{14}C]-epanolol, key and conditions as for Fig. 1.

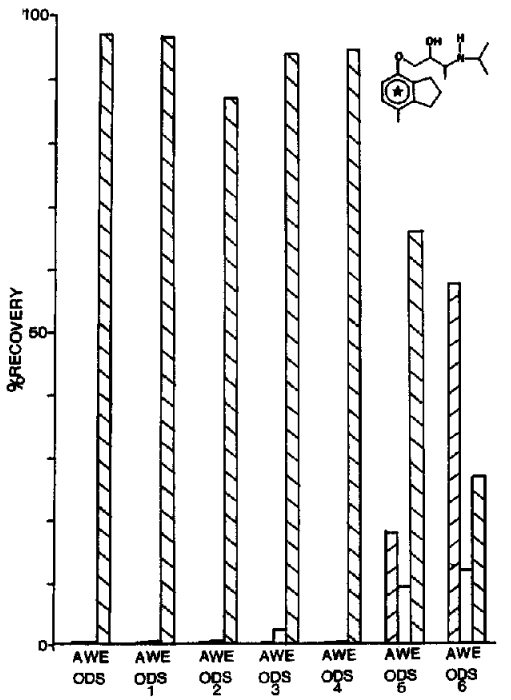

Figure 2
Recovery profile for [^{14}C]-ICI 118,551, key and conditions as for Fig. 1.

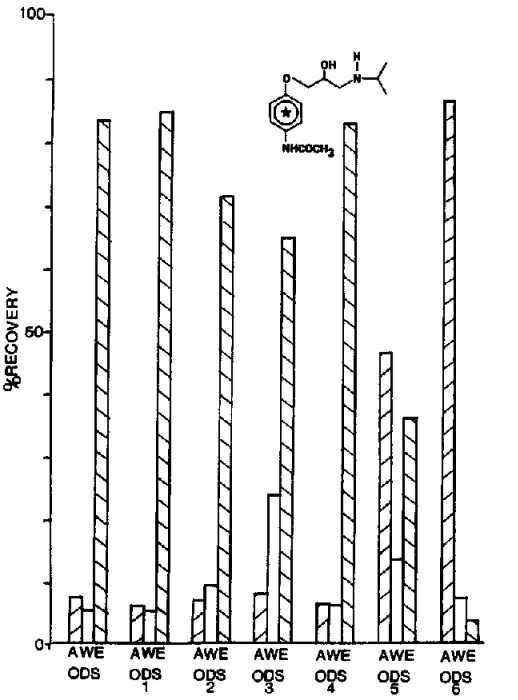

Figure 4
Recovery profile for [^{14}C]-practolol, key and conditions as for Fig. 1.

aminoethyl}-4-hydroxyphenylacetamide), specific activity 13.1 μCi mg^{-1} (structures are given as insets in Figs 1–4). All four compounds were extracted from both aqueous buffer solutions and dog plasma as described below.

Cartridges

The ODS (5% carbon load (CL)), ODS1 (14% CL), ODS2 (16% CL), ODS3 (10.5% CL, end capped (EC)), ODS4 (14% CL, EC), ODS5 (18% CL, EC), and ODS6 (22% CL, EC) C_{18} bonded SPE cartridges used in this study were obtained from Whatman Scientific (Maidstone, Kent, UK). Each 1 ml cartridge contained 100 mg of adsorbent.

Extraction procedure

Methanolic solutions (1 mg ml^{-1}) of the test compounds were spiked into either 0.2 M sodium acetate buffer (pH 5), or dog plasma–sodium acetate buffer (pH 5) (1:1, v/v) to give samples with a final concentration of 5 μg ml^{-1}. This concentration was chosen to ensure that sufficient of the radiolabel was present for efficient scintillation counting following extraction. Samples (1 ml) were applied to the cartridges which had previously been conditioned by sequential washing with methanol (2 × 1 ml), water (1 ml) and 0.2 M sodium acetate (pH 5, 1 ml). Following sample application the cartridges were washed with water (0.5 ml) and acetonitrile (0.5 ml). Adsorbed

Table 1
Solid-phase extraction of propranolol onto C_{18} bonded silica gel from aqueous buffer and dog plasma*

SPE phase	Sample	Application	Wash	Elution	MeOH/NaOH	Total
ODS	Buffer	0.10	0.65	99.63	0.08	
		0.12	0.58	99.43	0.68	
		0.11	**0.62**	**99.53**	**0.38**	100.64
	Plasma	1.01	0.30	99.11	0.15	
		1.26	0.29	102.84	0.28	
		1.14	**0.30**	**100.98**	**0.22**	102.62
ODS 1	Buffer	0.04	0.61	98.43	0.26	
		0.04	0.60	98.94	0.36	
		0.04	**0.61**	**98.69**	**0.31**	99.65
	Plasma	0.79	0.59	100.90	0.24	
		0.78	0.60	101.47	0.23	
		0.79	**0.60**	**101.19**	**0.23**	102.80
ODS 2	Buffer	0.04	0.62	91.70	1.08	
		0.05	0.67	80.27	0.99	
		0.05	**0.65**	**85.99**	**1.04**	87.73
	Plasma	1.37	0.84	78.19	1.17	
		1.35	0.82	78.85	0.83	
		1.36	**0.83**	**78.52**	**1.00**	81.87
ODS 3	Buffer	0.04	1.80	95.03	0.65	
		0.04	4.09	91.38	1.33	
		0.04	**2.95**	**93.21**	**0.99**	97.19
	Plasma	0.99	5.27	92.66	0.09	
		0.65	7.63	95.00	0.30	
		0.82	**6.45**	**93.83**	**0.20**	101.30
ODS 4	Buffer	0.14	0.66	97.47	0.33	
		0.15	0.63	98.60	0.27	
		0.15	**0.65**	**98.04**	**0.30**	99.14
	Plasma	1.90	0.75	98.11	0.24	
		0.83	0.77	100.83	0.23	
		1.14	**0.76**	**99.49**	**0.24**	101.83
ODS 5	Buffer	15.76	12.79	65.32	1.20	
		16.61	14.02	62.97	0.60	
		16.19	**13.41**	**64.15**	**0.90**	94.64
	Plasma	35.49	3.40	62.22	0.17	
		33.66	2.77	63.88	0.21	
		34.58	**3.09**	**63.05**	**0.19**	100.90
ODS 6	Buffer	36.24	18.30	41.67	0.70	
		55.67	15.17	23.97	0.37	
		45.96	**16.74**	**32.82**	**0.54**	96.06
	Plasma	62.60	6.69	34.78	0.15	
		61.99	2.25	26.61	0.15	
		62.30	**4.47**	**30.70**	**0.15**	97.53

*Figures in bold in this, and subsequent tables, are means.

compounds were recovered from the cartridges by elution with methanol–triethylamine acetate (0.1 M, pH 7) (80:20, v/v, 2 × 1 ml). In cases where an incomplete recovery of the radiolabel was obtained the cartridges were eluted with methanol–sodium hydroxide (0.1 M) (1:1, v/v, 2 × 1 ml).

Radioactivity in eluates was determined using scintillation counting on either a Beckman LS 5801 or Packard Tricarb 1900CA scintillation counter. Samples were mixed with 10 ml of Beckman 'Ready Value' scintillation fluid prior to analysis.

Results and Discussion

As described below the various different types of phase, with their variations in carbon loading and endcapping, were investigated for their ability to extract the four β-blockers from both aqueous buffer and dog plasma. In all cases the amount of radioactivity was measured in the column eluates following sample application in order to determine the initial extraction efficiency. The cartridges were then subjected to an acetonitrile wash to recover material being retained essentially by non-polar interactions with the C_{18} phase. Finally material retained by an ionic interaction with residual silanols was eluted with methanol–triethylamine acetate.

Effect of carbon loading on the extraction of β-blockers from buffer

In the absence of endcapping. The effect of differences in the carbon loading of the three

Table 2
Solid-phase extraction of ICI 118,551 onto C_{18} bonded silica gel from aqueous buffer and dog plasma

SPE phase	Sample	Application	Wash	Elution	MeOH/NaOH	Total
ODS	Buffer	0.09	0.18	99.14	0.34	
		0.05	0.18	96.14	0.36	
		0.07	**0.18**	**97.64**	**0.35**	98.24
	Plasma	0.13	0.20	97.73	0.16	
		0.14	0.20	98.11	0.17	
		0.14	**0.20**	**97.92**	**0.17**	98.43
ODS 1	Buffer	0.02	0.17	99.33	0.24	
		0.02	0.17	95.78	0.14	
		0.02	**0.17**	**97.56**	**0.19**	97.94
	Plasma	0.08	0.21	99.39	0.17	
		0.11	0.21	97.29	0.11	
		0.11	**0.21**	**98.34**	**0.14**	98.79
ODS 2	Buffer	0.05	0.20	88.86	0.52	
		0.02	0.25	86.40	0.52	
		0.04	**0.23**	**87.63**	**0.52**	88.42
	Plasma	0.15	0.28	89.94	0.41	
		0.09	0.27	95.82	0.28	
		0.12	**0.28**	**92.88**	**0.35**	93.63
ODS 3	Buffer	0.03	0.88	95.55	0.47	
		0.03	3.41	93.44	0.34	
		0.03	**2.15**	**94.50**	**0.41**	97.09
	Plasma	0.11	5.95	91.06	0.37	
		0.10	2.82	95.95	0.40	
		0.11	**4.39**	**93.51**	**0.39**	98.40
ODS 4	Buffer	0.02	0.03	97.09	0.23	
		0.03	0.19	92.78	0.23	
		0.03	**0.25**	**94.94**	**0.23**	95.45
	Plasma	0.71	0.22	96.43	0.32	
		1.46	0.46	94.58	0.39	
		1.09	**0.34**	**95.51**	**0.36**	97.30
ODS 5	Buffer	33.95	3.20	56.89	NR	
		1.90	15.08	75.62	NR	
		17.93	**9.14**	**66.26**	—	93.33
	Plasma	44.40	13.61	39.09	0.65	
		36.62	11.04	54.20	0.11	
		38.51	**12.33**	**46.65**	**0.38**	97.87
ODS 6	Buffer	64.06	12.49	19.25	0.36	
		51.66	10.31	32.83	—	
		57.86	**11.40**	**26.04**	**0.36**	95.66
	Plasma	30.44	9.38	53.79	0.70	
		5.80	4.54	83.78	0.41	
		18.12	**6.96**	**68.79**	**0.56**	94.43

unendcapped SPE phases was initially investigated by the extraction of the four test β-blockers from aqueous buffer. The results of these experiments are shown in Tables 1–4 and illustrated in Figs 1–4. As can readily be discerned from these data, increasing the carbon loading from 5% (ODS) to 14% (ODS1) and 16% (ODS2) had little effect on either the extraction, acetonitrile wash or methanol–triethyamine acetate elution steps. Typically for these compounds strong sorption was observed, with the acetonitrile wash causing little elution of radiolabel. The inability of a highly eluotropic solvent such as acetonitrile to elute these compounds provides good evidence for an ionic interaction between the analytes and silanols on the C_{18} bonded silica gel. Good recoveries were obtained at the elution step. Thus, for ICI 118551 and propranolol very little of the radiolabel was recovered in the eluates from the application and wash steps on any of the three phases examined, whilst good overall recoveries (<90%) were obtained with the final elution. In the case of practolol some of the radiolabel (>5%) was not retained on application, and a further quantity of the applied radiolabel (>5%) was recovered following the acetonitrile wash. Small losses at the acetonitrile wash step were also noted with epanolol. Overall recoveries for all four compounds were high (*ca* 90%). However, it is clear from the results presented in Table 1 that, despite these small variations in extraction properties between analytes, the extraction and elution profiles were hardly affected by the carbon

Table 3
Solid-phase extraction of epanolol onto C_{18} bonded silica gel from aqueous buffer and dog plasma

SPE phase	Sample	Application	Wash	Elution	MeOH/NaOH	Total
ODS	Buffer	2.43	5.08	90.46	1.01	
		2.73	5.11	87.41	0.61	
		2.58	**5.10**	**88.94**	**0.81**	97.43
	Plasma	3.54	4.56	91.80	0.48	
		4.10	4.15	90.68	0.42	
		3.82	**4.36**	**91.24**	**0.45**	99.88
ODS 1	Buffer	0.28	6.50	86.20	0.87	
		0.27	6.42	82.27	1.22	
		0.28	**6.46**	**84.24**	**1.05**	92.02
	Plasma	1.13	7.85	85.50	0.87	
		1.16	7.95	86.91	0.73	
		1.15	**7.90**	**86.21**	**0.80**	96.05
ODS 2	Buffer	0.26	6.61	86.66	1.02	
		0.25	6.54	85.55	0.96	
		0.26	**6.58**	**86.11**	**0.99**	93.93
	Plasma	1.65	11.36	85.71	0.56	
		0.69	8.64	91.65	0.15	
		1.17	**10.00**	**88.68**	**0.36**	100.21
ODS 3	Buffer	0.15	16.19	86.35	1.38	
		0.13	15.90	82.06	0.46	
		0.14	**16.05**	**81.21**	**0.92**	98.32
	Plasma	0.61	18.25	82.82	0.25	
		0.60	15.94	85.69	0.18	
		0.61	**17.10**	**84.26**	**0.22**	101.19
ODS 4	Buffer	0.12	6.74	85.91	0.43	
		0.11	6.54	85.28	0.54	
		0.12	**6.64**	**85.60**	**0.49**	92.85
	Plasma	0.64	7.31	91.98	0.36	
		0.72	6.93	92.35	0.32	
		0.68	**7.12**	**92.17**	**0.34**	100.31
ODS 5	Buffer	9.69	14.37	70.81	1.64	
		12.23	15.19	68.14	1.14	
		10.96	**14.78**	**69.48**	**1.39**	96.61
	Plasma	36.85	5.84	57.63	0.37	
		39.57	5.54	56.09	0.28	
		38.21	**5.69**	**56.86**	**0.33**	101.09
ODS 6	Buffer	71.81	21.55	5.17	0.24	
		61.15	20.09	16.24	0.31	
		66.48	**20.82**	**10.71**	**0.28**	98.28
	Plasma	90.30	7.34	7.55	0.13	
		89.64	4.75	11.18	0.21	
		89.97	**6.05**	**9.37**	**0.17**	105.56

Table 4
Solid-phase extraction of practolol onto C_{18} bonded silica gel from aqueous buffer and dog plasma

SPE phase	Sample	Application	Wash	Elution	MeOH/NaOH	Total
ODS	Buffer	8.58	5.06	85.74	0.43	
		8.42	5.67	82.79	0.40	
		8.50	**5.37**	**84.27**	**0.42**	98.56
	Plasma	9.76	5.65	84.72	0.33	
		9.65	5.86	83.40	0.43	
		9.71	**5.76**	**84.06**	**0.38**	99.91
ODS 1	Buffer	5.93	5.66	87.09	0.58	
		6.10	5.02	85.63	0.60	
		6.02	**5.34**	**86.36**	**0.59**	98.31
	Plasma	6.78	6.18	86.50	0.44	
		6.68	5.87	86.84	0.54	
		6.73	**6.03**	**86.67**	**0.49**	99.92
ODS 2	Buffer	6.90	7.60	78.34	0.76	
		7.28	11.67	76.19	0.62	
		7.09	**9.64**	**77.27**	**0.69**	94.69
	Plasma	8.14	13.13	74.09	0.84	
		10.44	11.64	71.13	1.57	
		9.29	**12.39**	**72.61**	**1.21**	95.50
ODS 3	Buffer	7.51	21.60	70.66	0.37	
		9.04	26.65	60.73	0.41	
		8.28	**24.13**	**65.70**	**0.39**	98.50
	Plasma	8.75	25.15	65.59	0.38	
		8.97	24.76	65.31	0.28	
		8.86	**24.96**	**65.45**	**0.33**	99.60
ODS 4	Buffer	7.01	6.24	84.52	0.50	
		6.38	6.73	83.29	0.57	
		6.70	**6.49**	**83.91**	**0.54**	97.64
	Plasma	7.72	7.62	84.27	0.50	
		7.08	7.77	85.39	0.59	
		7.18	**7.70**	**84.83**	**0.55**	100.26
ODS 5	Buffer	48.32	12.94	36.43	0.38	
		45.71	14.23	37.27	0.37	
		47.02	**13.59**	**36.85**	**0.38**	97.46
	Plasma	83.03	4.36	10.60	0.12	
		84.96	3.74	9.57	0.17	
		84.00	**4.05**	**10.09**	**0.15**	98.29
ODS 6	Buffer	86.99	7.07	3.57	0.08	
		87.99	6.66	4.27	0.12	
		87.49	**6.87**	**3.92**	**0.10**	98.38
	Plasma	93.72	3.46	3.87	0.08	
		91.87	3.34	3.81	0.08	
		92.80	**3.40**	**3.84**	**0.08**	100.12

loading of the SPE phases over the range examined. These results are similar to those obtained in our previous studies on C_{18} bonded phases from a range of manufacturers [1–3].

With endcapping. The effect of increasing carbon loading combined with endcapping to reduce the number of residual silanols on the extraction of the four test β-blockers was investigated concomitantly with the studies on the unendcapped material. In the case of the ODS3 and ODS4 (carbon loadings of 10.5 and 14%, respectively) the results for extraction from aqueous buffer were similar to those seen with the unendcapped materials. Such differences as were noted included an increase in the amount of epanolol and practolol recovered in the eluent from the acetonitrile wash step for the ODS3 material. However, this result was not repeated for the ODS 4 phase where amount of these substances recovered by the acetonitrile wash was similar to that found for the ODS, ODS1 and ODS2 materials. These results are given in Tables 1–4 and illustrated in Figs 1–4.

The results for the two endcapped phases with the highest carbon loadings (ODS5 and ODS6, 18 and 22%, respectively) showed very large differences compared to the other phases examined in this study. Thus significant amounts of radioactivity were detected in the eluents at the sample application step indicating a poorer, and more variable, extraction of all the test compounds. In further contrast to the other phases significant amounts of radiolabel were also recovered in the eluates from

the acetonitrile wash step. As can be seen in Table 2 the higher the carbon loading, when combined with endcapping, the poorer the initial extraction. For example, in the case of propranolol *ca* 9% of the radiolabel was unretained on the ODS5 SPE column, and a further *ca* 7% was eluted with acetonitrile. On the ODS6 column *ca* 46% of the propranolol applied to the column passed through unretained with a further 17% recovered in the acetonitrile wash.

Effect of plasma on the extraction of β-blockers onto C_{18} bonded cartridges

Matrix effects are not an uncommon feature in sample preparation and we therefore examined whether the extraction profile of the test analytes was affected by changing from aqueous buffer to dog plasma. In general the extraction of all four compounds on the ODS, ODS1, ODS2, ODS3 and ODS4 phases was very similar to that seen from buffer. Once again, where differences were seen, they were most pronounced for the ODS5 and ODS6 cartridges. Thus in extreme cases, such as epanolol and practolol, virtually none of the radiolabel was retained on the column during the application step. Extraction onto these high carbon loading-endcapped phases was generally poorer from dog plasma compared to aqueous buffer.

Conclusions

It is quite clear that endcapping, when combined with a high carbon loading, had a profound effect on the extraction of the four β-blockers studied under the conditions employed here. Thus, the higher the carbon loading–endcapping the poorer the extraction and retention of the analytes. Matrix effects were evident and the application of the sample in plasma clearly exaggerated these trends.

In our previous work we had attributed the results that we obtained for the extraction of these compounds onto C_{18} bonded silica as being due to a mixture both a reversed-phase and a 'secondary', cation exchange, interaction with residual silanols. It seems clear from the present study that far from being a mere 'secondary' interaction the cation exchange mechanism is the primary reason for the extraction of basic analytes such as the β-blockers.

Irrespective of the mechanism the practical consequences of these observations are quite clear. In order to obtain high initial extraction efficiencies onto C_{18} bonded SPE phases for analytes such as the β-blockers, materials with a relatively low carbon loading and without endcapping should be selected. It may be that at least some of the variability in extraction efficiency noted for different batches of cartridges may be attributable to different degrees of coverage of the silica. Indeed it might be argued that extraction of such compounds would be more reliably achieved on a cation exchange material rather than a reversed-phase silica.

Further studies, aimed at achieving a better understanding of the role of silanols in the extraction of basic compounds, and the investigation of the utility of cation and mixed mode SPE materials are continuing.

References

[1] D.W. Roberts, R.J. Ruane and I.D. Wilson, *J. Pharm. Biomed. Anal.* **7**, 1077–1086 (1989).
[2] P. Martin, B. Leadbetter and I.D. Wilson, *J. Pharm. Biomed. Anal.* **11**, 307–312 (1993).
[3] R.J. Ruane and I.D. Wilson, *J. Pharm. Biomed. Anal.* **5**, 723–727 (1992).
[4] 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).
[5] B. Law, S. Weir and N.A. Ward, *J. Pharm. Biomed. Anal.* **10**, 167–179 (1992).
[6] B. Law and S. Weir, *J. Pharm. Biomed. Anal.* **10**, 182–186 (1992).

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