Journal of Pharmaceutical & Biomedical Analysis
Vol. 5, No. 7, pp. 723–727, 1987
Printed in Great Britain

0731–7085/87 $3.00 + 0.00
Pergamon Journals Ltd.

Short Communication

The use of C18 bonded silica in the solid phase extraction of basic drugs — possible role for ionic interactions with residual silanols

R. J. RUANE* and I. D. WILSON

Department of Safety of Medicines, ICI Pharmaceuticals Division, Mereside, Alderley Park, Macclesfield, Cheshire, UK

Keywords: *Solid-phase extraction; C18 bonded silica; basic drugs; liquid–solid extraction.*

Introduction

It is rarely possible to analyse biological fluids such as plasma and urine without some form of sample preparation. This usually involves concentrating the compound of interest, selectively removing endogenous sources of interference and presenting the sample in a suitable form for chromatography. Devising suitable procedures of sample preparation is therefore a critical and often time-consuming step in the development of an analytical method. One of the major innovations in recent years has been the introduction of systems for solid-phase (SPE) or liquid–solid (LSE) extraction whereby the analyte is selectively adsorbed from the biological matrix on to one of a variety of commercially available solid phases (e.g. silica or bonded silica) contained within a disposable cartridge [1]. By careful selection of suitable washing and elution schemes it is often possible to obtain samples for subsequent analysis which contain very few interfering substances; thus sensitive and specific assays can be devised.

The results described in the present communication were obtained during studies aimed at devising suitable SPE methods for the assay of a number of basic drugs in plasma. All the compounds of interest (Fig. 1) had in common a secondary amino-function connected via a methylene bridge to a secondary hydroxyl group, that is a side-chain typical of beta-blocker drugs like propranolol. Although the extraction was developed on a C18 bonded silica where a reversed-phase mechanism might be expected to predominate, the results suggested that other factors may be more important. Furthermore there were significant differences between C18 cartridges nominally of the same type but obtained from different manufacturers.

*To whom correspondence should be addressed.

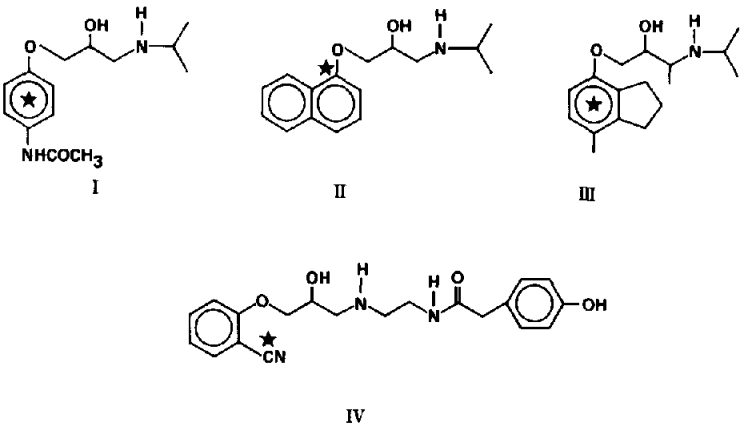

Figure 1
Structures of beta blocker compounds. I: ICI 50,172 (Practolol): specific activity 8.8 μCi/mg; (2*RS*)-3-(4-acetamidophenoxy)-1-isopropylamino-2-propanol; II: ICI 45,520 (Propranolol): specific activity 38.4 μCi/mg; (2*RS*)-1-isopropylamino-3-(1-naphthyloxy)-2-propanol; III: ICI 118,551: specific activity 10.6 μCi/mg; (2*RS*,3*RS*)-1-(7-methylindan-4-yloxy)-3-isopropylaminobutan-2-ol; IV: ICI 141,292: specific activity 13.1 μCi/mg; *N*-(2-[(2*RS*)-3-*o*-cyanophenoxy-2-hydroxypropyl]aminoethyl)-4-hydroxyphenylacetamide. ★: denotes position of radiolabel.

Experimental

Materials

The ^{14}C-radiolabelled beta-blocker compounds (Fig. 1, I–IV) of radiochemical purity >95% were synthesised in the radiochemical laboratory at ICI Pharmaceuticals Division (Mereside, Alderley Park, Macclesfield, Cheshire, UK). The Bond Elut^{TM} C18 and silica gel solid-phase extraction cartridges (1 ml) were manufactured by Analytichem International (Harbor City, California, USA) and the SPE^{TM} C18 cartridges (1 ml) by J. T. Baker (Phillipsburg, New Jersey, USA). All solvents were of HPLC grade and other reagents were of Analar grade.

Cartridge preparation

The C18 cartridges were prepared for use by washing with methanol (2 × 1 ml), then water (1 ml) and finally with 0.2 M sodium acetate (adjusted to pH 5.0 with glacial acetic acid) (1 ml). Silica gel cartridges were prepared in the same manner.

Sample preparation

Samples for extraction were prepared by mixing dog plasma (0.5 ml) and sodium acetate (pH 5.0) (0.5 ml) and then spiking with the appropriate radiolabelled beta-blocker (10 μg in methanol).

The spiked samples were then applied to the activated cartridges and washed with water (1 ml) and then with acetonitrile (0.5 ml). Analytes were then recovered from the cartridges with methanol–0.1 M triethylamine acetate (pH 7.0) (80:20, v/v) (2 × 1 ml). Radioactivity in the various eluates was determined by liquid scintillation counting (LSC).

Results and Discussion

All the compounds were efficiently extracted from spiked dog plasma using the Bond Elut C18 cartridges. With the Baker C18 cartridges the efficiency of extraction from plasma was generally only about 80%, but was less than 60% for compound I. Both types of cartridge were then washed with acetonitrile, a strongly eluotropic solvent which would normally be expected to provide the ultimate step to such an extraction scheme since the compounds of interest would be expected to be recovered in the eluate. However, somewhat surprisingly, the recovery of radiolabel from the Bond Elut C18 cartridges with this solvent was very poor (less than 20%) as shown in Table 1. Recoveries from the Baker C18 cartridges were somewhat higher (10–40%) but were nevertheless much lower than those that might have been expected if a conventional reversed-phase mechanism was operating. Good recoveries of adsorbed material were obtained when methanol–triethylamine acetate (pH 7.0) was used as eluent. Use of a simple unbuffered methanol–water eluent was insufficient to obtain good recoveries. Such results were strongly indicative of an ionic interaction between the basic (positively charged) drug molecules and the residual (negatively charged) silanol groups remaining on the surface of the silica gel after silylation, rather than an interaction with the C18 phase. Support for this hypothesis comes from experiments whereby one of the compounds (III) was shown to be adsorbed on to a silica Bond Elut cartridge from both aqueous and methanolic solution and was then removed using methanol–triethylamine acetate.

Differences between the two types of C18 bonded materials, including the less efficient extraction of some of the compounds from plasma by the Bond Elut C18 column are therefore probably explained in terms of the degree of coverage with C18 groups and the extent of end-capping of the residual silanols. Whatever the reason for the observed differences, the phenomenon can be easily and reproducibly exploited for the selective extraction of beta-blocker drugs. The ability to wash the C18 cartridges with a strongly eluotropic solvent to remove the bulk of the endogenous contaminants followed by recovery of the compound of interest, as has been described, provides the basis for a highly specific and selective assay.

However, it should be noted that although all four compounds show similar behaviour under the conditions described, there are differences. For example, on the whole, compounds I and II are not as efficiently retained on the cartridges as are III and IV. Clearly there would be considerable benefit if these differences could be explained on the basis of some easily determined physicochemical property such as pK_a or log P. However, there does not appear to be a relationship between either pK_a or log P and the observed results. For example, as mentioned above, on the Bond Elut cartridges compounds I and II and compounds III and IV form 2 groups; the last two compounds are the best retained. The pK_a values for compounds I to III are very similar (Table 2) whereas that for compound IV is quite different. Similarly log P does not seem to be a useful predictor of behaviour for SPE since compounds I and IV (also II and III) have similar values for this parameter but show differences in extraction.

Similarly, there appears to be no correlation between log P or pK_a and the results for the Baker C18 cartridges. The picture is further complicated since compounds I to III show a similar pattern of extraction on to both Bond Elut and Baker cartridges; however, compound IV like compound III is well retained on the Bond Elut cartridge but behaves more like compounds I and II on the Baker cartridge and is poorly retained.

Table 1
Recovery of radioactivity expressed as a percentage of the total applied to the C18 cartridges when washed with acetonitrile and then with methanol–0.1 M triethylamine acetate (pH 7.0) (80:20, v/v)*

Compound	Recovery of radioactivity (%) (mean ± SD, $n = 3$) Acetonitrile		Methanol–triethylamine acetate (80:20, v/v)		Overall total recovery	
	Bond Elut C18 cartridges mean ± SD	J. T. Baker C18 cartridges mean ± SD	Bond Elut C18 cartridges mean ± SD	J. T. Baker C18 cartridges mean ± SD	Bond Elut cartridges	J. T. Baker cartridges
I ICI 50,172	10.3 ± 0.9	36.5 ± 1.1	93.7 ± 0.5	18.8 ± 0.8	104 ± 0.6	55.3 ± 0.7
II ICI 45,520	17.0 ± 0.6	31.6 ± 2.4	77.3 ± 0.5	48.0 ± 4.1	94.2 ± 0.8	79.6 ± 2.0
III ICI 118,551	0.6 ± 0.1	11.5 ± 3.9	94.3 ± 2.5	78.3 ± 6.5	95.1 ± 2.8	89.7 ± 2.9
IV ICI 141,292	2.8 ± 0.4	39.7 ± 2.0	100.2 ± 1.7	40.0 ± 1.1	103 ± 2.0	79.7 ± 0.9

*The low overall recoveries of radioactivity seen for the J. T. Baker C18 cartridges are due to non-retention of radiolabel during the application step.

Table 2
Log *P* and pK_a values for compounds I–IV

Compound		log *P*	pK_a
I	ICI 50,172	0.79	9.50
II	ICI 45,520	3.56	9.45
III	ICI 118,551	3.82	9.56
IV	ICI 141,292	0.87	7.72

Such differences between the cartridges probably result from different production methods and may reflect the extent and type of coverage with the C18 phase and the number of residual silanols remaining after manufacture. Further work is now in progress to supplement these preliminary results and extend the experiments to include an examination of the effects of structure of the compound, organic modifier, buffer, pH, solvent strength and stationary phase (C2, C8, phenyl, etc.). From such studies a more complete explanation of the observations in the present work may be possible.

The differences noted between the two C18 bonded silicas from these manufacturers illustrate the need for caution when the transfer of an extraction scheme from one product to another is contemplated. As shown in the present work nominally similar products had quite different properties under the conditions employed for this structurally related group of compounds. It seems logical to assume that, at least in part, the effects observed were due to the presence of residual silanols on the C18 bonded silica. Certainly the interaction of basic drugs with silanols on incompletely end-capped silica gel is a widely acknowledged problem in reversed-phase HPLC where it is often the cause of severe peak tailing [2–4]. In the particular examples described in the present work the assumed presence of residual silanols on the C18 cartridges (particularly the Bond Elut material) was in fact advantageous. In other applications this might not be the case; thus, the differences observed between the two products may provide a valuable source of selectivity.

Conclusion

Solid-phase extraction is being used increasingly to replace conventional liquid–liquid extraction. However, the former technique provides a much more complex system where several factors may be responsible for the observed results. By comparison liquid–liquid extraction is usually simply a matter of balancing the lipophilicity of the analyte against the polarity of the extracting solvent. The ability to exploit subtle "secondary" interactions of the type observed in these preliminary experiments should allow the use of LSE to perform sample pretreatment that would not be possible using simple solvent extraction.

References

[1] R. D. McDowall, J. C. Pearce and G. S. Murkitt, *J. Pharm. Biomed. Anal.* **4**, 3–21 (1986).
[2] N. C. Cooke and K. Olsen, *Am. Lab. Magazine* **11** (8), 45–60 (1979).
[3] I. M. Johanson, K. G. Wahlund and G. J. Schill, *J. Chromatogr.* **149**, 281–296 (1978).
[4] K. G. Wahlund and A. Solowski, *J. Chromatogr.* **151**, 299–310 (1978).

[*First received for review 10 March 1987; revised manuscript received 29 April 1987*]