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The use of graphitized carbon black in solid phase extraction: comparison with C_{18} bonded silica gel*

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Abstract: Preliminary studies on the properties of a commercially available graphitized carbon black for the solid-phase (or liquid–solid) extraction of drugs and metabolites from biological fluids such as urine and plasma are described. A variety of basic drugs, all of the β-blocker type, were efficiently extracted from dog plasma and subsequently recovered from the adsorbent using chloroform–methanol mixtures. Similarly metabolites of the non-steroidal anti-flammatory drug ibuprofen also were efficiently extracted from human urine. The proportions of methanol and chloroform used for elution were found to be critical to ensure complete recoveries of adsorbed material.

Proton NMR studies of the column eluates from both carbon and C_{18} bonded silica gel extraction cartridges revealed differences in capacity and selectivity following the application of ibuprofen containing metabolite samples.

Keywords: *Solid-phase extraction; urine; plasma; β-blockers; ibuprofen; graphitized carbon black.*

Introduction

Sample preparation often forms a critical part of methods devised for the trace analysis of drugs and metabolites present in complex matrices, such as plasma and urine. Indeed in the absence of effective sample pretreatment the specific quantitative analysis of substances present at concentrations in the ng or μg ml^{-1} range in biological samples is often impractical by chromatographic methods. In the broadest sense, the aims of sample preparation can be defined as the selective concentration of the compounds of interest, the removal of the bulk of the endogenous contaminants, the elimination of interferences and the presentation of the sample in a suitable form for subsequent chromatographic analysis. To these it should perhaps be added that sample preparation needs to be performed as rapidly and efficiently as possible.

The introduction of solid-phase extraction (SPE) systems (or liquid–solid extraction, LSE) represented a major innovation in sample preparation allowing not only extraction

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but also analyte concentration to be accomplished in a single step. Many solid-phase adsorbents are currently available, generally based on silica gel and its various alkyl modifications to give a variety of bonded phases (e.g. C_2, C_8, C_{18}, cyclohexyl, SAX, SCX, etc.). These materials, contained within a disposable cartridge, are used to selectively extract the compounds of interest from the sample onto the solid phase from which they are subsequently eluted using a suitable solvent. The combination of an appropriate SPE phase and careful selection of elution solvent(s) can enable highly selective assays to be developed.

Initially SPE methodology was slow to gain acceptance, due in part to the perceived expense of the cartridges compared to traditional techniques such as liquid–liquid extraction. However, SPE is now an accepted technique which, because of its convenience, flexibility and relative ease of automation, is rapidly displacing solvent extraction in many laboratories.

To date the bulk of the available SPE packings have been silica based materials similar to those used as stationary phases. However, despite the proven usefulness of silica based phases in both high-performance liquid chromatography (HPLC) and SPE there is no fundamental reason why other materials should not be used in the latter application. Indeed, it is quite possible that certain advantages might result from the use of non-silica based SPE phases. Accordingly, in common with a number of other groups [1–3], an investigation of the use of graphitized carbon black as an adsorbent for the analysis of drugs and their metabolites present in biological fluids was initiated. Here preliminary results are presented for the extraction of a range of drugs and drug metabolites from urine and plasma using both carbon and C_{18} bonded silica gel.

Experimental

Materials

The ^{14}C-radiolabelled β-blocker compounds (Fig. 1) **I–IV**: **I**, Practolol (ICI 50,172), specific activity 8.8 μCi mg^{-1}, (2RS)-3-(4-acetamidophenoxy)-1-isopropylamino-2-propanol; **II**, Propranolol (ICI 45,520), specific activity 38.4 μCi mg^{-1}, (2RS)-1-isopropylamino-3-(1-naphthyloxy)-2-propanol; **III** (ICI 118,551), specific activity

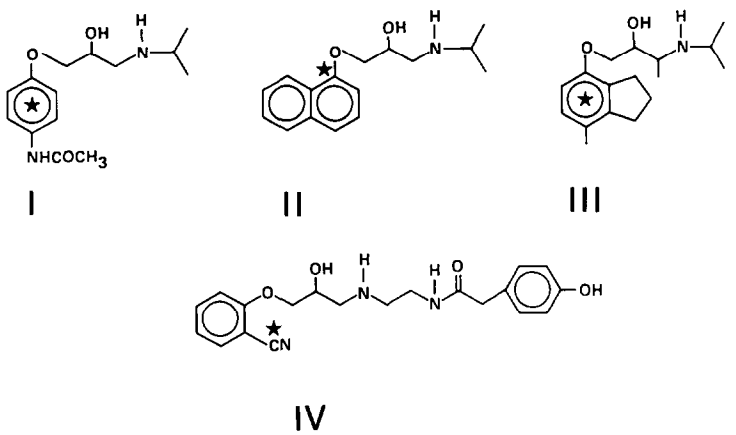

Figure 1
Structure of the four "β-blockers" used in these studies: **I**, ICI 50,172; **II**, ICI 45,520; **III**, ICI 118,551; **IV**, ICI 141,292.

10.6 μCi mg^{-1}, (2RS,3RS)-1-(7-methylindan-4-yloxy)-3-isopropylaminobutan-2-ol; **IV** (ICI 141,292), specific activity 13.1 μCi mg^{-1}; *N*-{2-[(2RS)-3-*o*-cyanophenoxy-2-hydroxypropyl]aminoethyl}-4-hydroxyphenylacetamide, used in this study were all synthesized in the radiochemical laboratory at ICI Pharmaceuticals (Alderley Park, Cheshire, UK) and had a radiochemical purity of >95%. Ibuprofen, 2-(-isobutylphenyl) propionic acid, was obtained from Boots Ltd (Nottingham, UK) and was used as supplied.

The Bond Elut™ C_{18} SPE cartridges (1 and 3 ml) manufactured by Analytichem International (Harbor City, CA, USA) were supplied by Jones Chromatography Ltd (Hengoed, Mid Glamorgan, Wales). C_{18} SPE™ cartridges (1 ml) manufactured by J. T. Baker (Phillipsburg, NJ, USA), Sep Pak™ cartridges by Millipore/Waters (Milford, MA, USA), C_{18} Adsorbex™ cartridges (3 ml) manufactured by E. Merck Ltd (Darmstadt, FRG) were supplied by BDH Ltd (Poole, Dorset, UK), C_{18} SPE-ED™ cartridges manufactured by Applied Separations (Bath, PA, USA) were supplied by Laboratory Impex Ltd (Teddington, UK). The 3 ml C_{18} Supelclean™ cartridges and 3 ml Carbopack cartridges manufactured by Supelco Ltd (Bellefonte, PA, USA) were supplied by Radleys Ltd (Sawbridgeworth, Herts, UK). For some experiments the Carbopack material was repacked into 1 ml cartridges (containing 100 mg of material). All solvents were of HPLC grade whilst other reagents were of Analar grade.

Cartridge preparation

β-*Blocker compounds*. The C_{18} (1 ml) cartridges were prepared 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).

The 1 ml, repacked, graphitized carbon black cartridges were prepared by washing sequentially with chloroform (2 × 1 ml), methanol (2 × 1 ml) and water (2 × 1 ml). In the case of the 3 ml cartridges solvent volumes were doubled.

Ibuprofen metabolites. The C_{18} (1 and 3 ml) cartridges generally were prepared by washing with methanol containing 1% (v/v) of 98% formic acid (3 ml), followed by water, also containing 1% (v/v) of 98% formic acid (3 ml).

For most experiments the graphitized carbon cartridges (repacked 1 and 3 ml) were prepared by washing sequentially with chloroform (3 ml), methanol containing 1% (v/v) formic acid (3 ml) and water also containing 1% (v/v) formic acid (1 ml).

For some experiments acidified solvents were replaced by solvents from which formic acid had been omitted (see text).

Samples and sample preparation

β-*Blocker compounds*. Samples for extraction onto the C_{18} columns were prepared by mixing dog plasma (0.5 ml) and 0.2 M sodium acetate, pH 5.0 (0.5 ml) and then spiking with the appropriate radiolabelled β-blocker (10 μg) in methanol (10 μl). The spiked samples were then applied to the activated cartridges and washed with water (1 ml) followed by acetonitrile (0.5 ml). The radiolabelled compounds were eluted from the C_{18} phase using methanol/0.1 M triethylamine acetate, pH 7.0 (80:20, v/v, 2 × 1 ml). The radioactivity in the various eluates was subsequently determined by liquid scintillation counting.

In the case of the repacked 1 ml graphitized carbon phase the plasma samples (1 ml), containing 10 μg of radiolabelled compound, were applied directly to the activated

cartridge (see above). The cartridges were then washed sequentially with water (1 ml) followed by cyclohexane (0.5 ml). The radiolabelled β-blockers were recovered by elution with chloroform/methanol (80:20, v/v; 3 × 1 ml).

Where 3 ml Carbopack cartridges were used, the sample volume (1 ml of plasma plus 10 μg of compound) was not changed, but that of the washing and elution solvents were doubled.

The recovery of radioactivity in the various eluates was then determined using liquid scintillation counting.

Ibuprofen metabolites. Urine samples containing ibuprofen metabolites (ibuprofen glucuronide, 1G; its hydroxylated metabolite, 2-[-4-(2-hydroxy-2-methylpropyl)phenyl] propionic acid, HMPPP; and the diacid metabolite, 2-[4-(2-carboxy-2-methylpropyl) phenyl]propionic acid, CMPPP) were obtained in the 2 to 4 hr urine sample voided following a 400 mg dose of the drug to a normal healthy male subject (aged 35 years). The samples were acidified on collection to an approximate pH of 2, by the addition of 1% (v/v) of 98% formic acid, in order to stabilise the labile ester glucuronide conjugates of ibuprofen and its hydroxylated metabolite.

Samples of this urine (1 ml) were then applied to 1 or 3 ml Bond Elut C_{18} cartridges prepared as described above. Adsorbed material was recovered from the cartridges by washing with acidified methanol (containing 1%, v/v, of formic acid, 98%).

Similarly ibuprofen metabolite containing urine samples (1 or 2 ml) were applied to repacked 1 and 3 ml Carbopack cartridges, prepared as described for the β-blocker compounds. Adsorbed material was recovered from the cartridge using chloroform/methanol (1:1, v/v; 5 ml).

The eluates obtained from these urine samples, both non-retained and adsorbed material, were analysed by Fourier transform (FT) proton NMR. Aqueous eluates were reduced to dryness by freeze drying, whilst organic solvents were removed under a stream of nitrogen (residual water and solvent were also removed by freeze drying). Samples were then redissolved in either D_2O (1 ml) or D_6 DMSO (1 ml) (99.98 atom %, Aldrich Chemical Co. Ltd, Gillingham, UK).

Spectroscopy was performed on either a Bruker AM 200 NMR spectrometer, operating at 200 MHz, or a Bruker AC 250 NMR spectrometer, operating at 250 MHz. Both instruments were equipped with autosamplers. Spectra were the result of 512 free induction decays (FIDs). Data was collected into either 16K (200 MHz) or 32K data points (250 MHz).

The spectra were obtained at either ambient probe temperature (25°C; 250 MHz) or at 27°C (200 MHz). In order to suppress signals due to residual water protons a secondary irradiation field was applied at the resonance frequency of water (4.8 ppm).

Results and Discussion

Extraction of β-blockers from plasma

In previous studies on the SPE of the four β-blocker type compounds shown in Fig. 1 onto C_{18} bonded silica gel, from a variety of manufacturers, wide variations in the results were observed depending upon the particular phase, the test compound and on the elution conditions used [4, 5]. In general with these compounds it appeared that the C_{18} bonded phases were behaving more like weak ion-exchange columns, with high extraction efficiencies at a pH of 5 (where the secondary amine present in all four

compounds would have been in the ionized state) and poor recoveries from the cartridge using the aprotic solvent acetonitrile for elution. In order to explain this type of result it seemed logical to invoke secondary ionic interactions between the secondary amine present in all of these compounds and residual silanols, rather than a simple reversed-phase mechanism.

Such interactions are of course well known in the reversed-phase HPLC of such compounds and are responsible for the often poor chromatographic properties of these weak bases (characterized by distorted, badly tailing peaks). Some of the results obtained in these earlier studies are illustrated in Table 1 for the extraction of compound III (ICI 118,551), from dog plasma, onto a variety of C_{18} bonded phases. Clearly pure acetonitrile is a poor eluent for this compound on all six phases examined, whilst methanol/aqueous triethylamine acetate buffer (80:20) gives much higher recoveries. There were, however, two exceptions namely, the C_{18} phases produced by J. T. Baker and Supelco. In the case of the former much more material (over 10%) was eluted by acetonitrile, whilst an additional 10% of the total ICI 118,551 present in the sample was simply not retained under the loading conditions used. These results are consistent with the manufacturer's claims to have extensively end-capped residual silanols (although it is clearly not possible to completely eliminate them). In the case of the Supelco phase the poor overall recovery seen here is due to a very strong retention of 10% of the adsorbed material which required the use of 0.1 M sodium hydroxide/methanol (1:1, v/v) to ensure its elution. It should be noted that the similarity in results obtained for the other C_{18} phases with ICI 118,551 was not always reflected when the other β-blocker compounds were tested [4, 5]. Thus between different manufacturers' products there appear to be wide variations in the total amount of these "secondary" (i.e. non-bulk interaction) properties. This is unsurprising and presumably reflects differences in the silica support, the process of manufacture of the C_{18} phase and the extent and type of end-capping and is, of course, only important if an assay is developed which depends on the use of such interactions. These can be very useful and indeed we have exploited this phenomenon to develop a highly specific HPLC assay for ICI 118,551 and two of its metabolites (R. J. Ruane *et al.*, in preparation).

However, it does mean that, having developed such an assay, switching from one manufacturer's C_{18} phase to another could be problematic and is certainly something

Table 1
Recovery of ^{14}C-ICI 118,551 from C_{18} cartridges supplied by different manufacturers using acetonitrile and methanol/pH 7.0 triethylamine acetate (0.1 M; 80:20) as eluents

Cartridge	% Recovery ± SE (n = 3) Acetonitrile	MeOH/buffer	Total
Bond Elut	0.6 ± 0.1	94.3 ± 2.5	94.9
Baker	11.5 ± 3.9	78.1 ± 6.5	89.6
Waters SEP PAK	1.7 ± 0.7	93.8 ± 2.6	94.5
Merck Adsorbex*	0.4 ± 0.2	90.6 ± 1.5	91.0
Supelco	0.8 ± 0.2	88.2 ± 3.9	89.0
SPE-ED*	0.4 ± 0.1	94.0 ± 1.1	94.4

N.B. The low overall recovery of radioactivity seen for Baker cartridges is due to non-retention (breakthrough) of radiolabel during the application step rather than irreversible binding to the C_{18} phase. The low overall recovery for the Supelco phase is the result of very strong absorbtion (see text).

*3cc Cartridges were only available at the time of these experiments.

which requires caution. In addition, it also requires great batch to batch consistency by individual manufacturers if a method relying on silanol interactions is to be used over a long period of time. For extractions based on bulk interactions with the C_{18} phase such considerations are obviously much less likely to be important. However, our results illustrate the potential complexity and subtlety of SPE systems involving silica based chemistries and highlight the need for the analyst to thoroughly investigate the mechanism by which his extraction is operating. Whilst it was possible to exploit the presence of residual silanols on the C_{18} packings for the analysis of certain drugs, situations are envisaged where it would be advantageous to eliminate such interactions entirely. For this reason the authors were particularly interested by reports [1–3] of the use of graphitized carbon black (Carbopack) for SPE.

For efficient extraction of compounds from aqueous solutions (such as plasma or urine) it is first necessary to prewash the Carbopack with chloroform, methanol and finally water. This is analogous to the situation for C_{18} bonded SPE packings which are "activated" by prewashing with methanol (or some other suitable organic solvent), followed by water (or an aqueous buffer). These prewashing steps serve to wet the intensely hydrophobic phase and ensure that solvents present in the aqueous sample can fully interact with the stationary phase. Following these prewashing steps plasma samples containing 10 μg of each of the four radiolabelled β-blockers were applied to cartridges containing 100 mg of the Carbopack material.

In all cases, >90% of the radiolabel was retained (see Table 2). The columns were then washed with a small volume of water (to displace residual plasma remaining in the column voids) followed by cyclohexane (to remove water). The retained compounds were then eluted with a mixture of methanol and chloroform. Interestingly, attempted elution with either pure methanol or pure chloroform proved to be rather inefficient in eluting the adsorbed radiolabelled compounds (see also the results for the ibuprofen metabolites). High extraction efficiencies were achieved without the need to modify the pH of the plasma prior to application of the β-blockers and the importance of this parameter for this class of compounds was not investigated further (however see later results for ibuprofen metabolites).

Table 2
Recovery of ^{14}C-labelled β-blockers from Carbopack cartridges using chloroform/methanol (80:20, v/v) as eluent

Compound	% Recovery ± SD (n = 3)			
	Non-retained	Cyclohexane wash	Chloroform–methanol	Total
ICI 118,551	4.3 ± 1.8	0.5 ± 0.1	94.9 ± 2.7	99.7
ICI 45,520	6.7 ± 0.2	0.8 ± 0.1	95.0 ± 0.5	102.5
ICI 141,292	9.3 ± 2.1	ND	93.3 ± 3.4	102.6
ICI 50,172	3.3 ± 0.4	1.4 ± 2.3	93.9 ± 4.8	98.6

ND = >0.1%.

Extraction of ibuprofen metabolites from urine

Ibuprofen is metabolized by man to give three major urinary metabolites, an ester glucuronide of the parent compound (1G), as a side chain hydroxylated metabolite, HMPPP (also excreted as an ester glucuronide), and a side chain oxidized dicarboxylic acid, CMPP (for structures see Fig. 2, and for chemical names see Experimental). In Figure 2 a proton NMR spectrum of a freeze dried urine sample redissolved in D_2O,

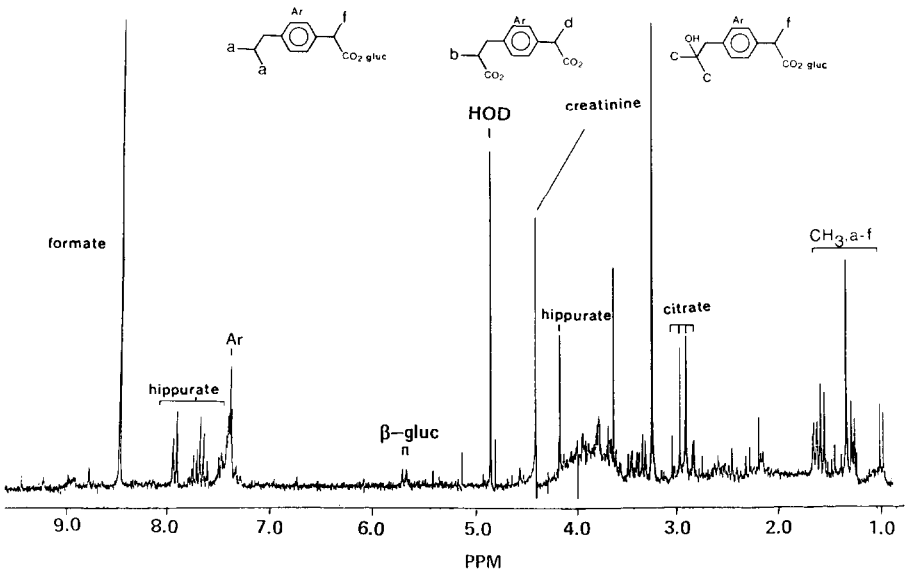

Figure 2
^{1}H NMR spectrum of freeze-dried human urine (in D_2O) containing ibuprofen metabolites (IG, HMPPP and CMPPP, see inset for structures) obtained for the period 2–4 h after an oral dose of 400 mg of ibuprofen. Signals for both endogenous compounds (e.g. hippurate, creatinine, etc.) are present, whilst a number of prominent resonances from ibuprofen metabolites are also visible (side chain methyls 1 to 2 ppm, β-anomeric proton of glucuronides ~5.5 ppm, aromatic protons ~7.4 ppm). More detailed interpretation is given in refs 7 and 8.

obtained for a sample covering the period 2–4 h after a single oral dose of 400 mg of ibuprofen is shown. Signals resulting from the various ibuprofen metabolites are readily apparent in the aromatic and aliphatic portions of the spectrum, together with signals resulting from the major endogenous urinary components (e.g. citrate, succinate, hippurate, creatinine, etc.). This, and subsequent figures, illustrate one of the major advantages resulting from the use of NMR to study SPE.

This is that, not only can the components of interest be followed relatively easily, but the nature and identity of co-eluting contaminants also can be determined (as indeed can the components removed during sample preparation). It is difficult to envisage how information on such a structurally diverse range of compounds would be obtained as simply using chromatography based methodology (for other examples of the use of NMR combined with SPE see refs 6–8). Thus, in many ways the urine sample illustrated in Fig. 2 can be considered to be a multi-component "test mixture" containing a variety of polar acidic, basic and amphoteric components in addition to the metabolites of ibuprofen.

In Figs 3A and 3B spectra of the material eluted from a 1 ml C_{18} Bond Elut^{TM} column (containing 100 mg of adsorbent) using pure methanol and that recovered from a 1 ml (100 mg) repacked Carbopack cartridge using chloroform/methanol (1:1, v/v), respectively are shown. Many similarities are apparent between the two extracts. For example both spectra contain strong resonances for the three ibuprofen metabolites, together with signals due to hippurate. In both cases an examination of the spectra obtained for the non-retained portion of the sample showed that extraction of these components was complete. An interesting difference between the C_{18} phase and the carbon packing was the appearance of prominent signals for creatinine in the chloroform–methanol eluate of

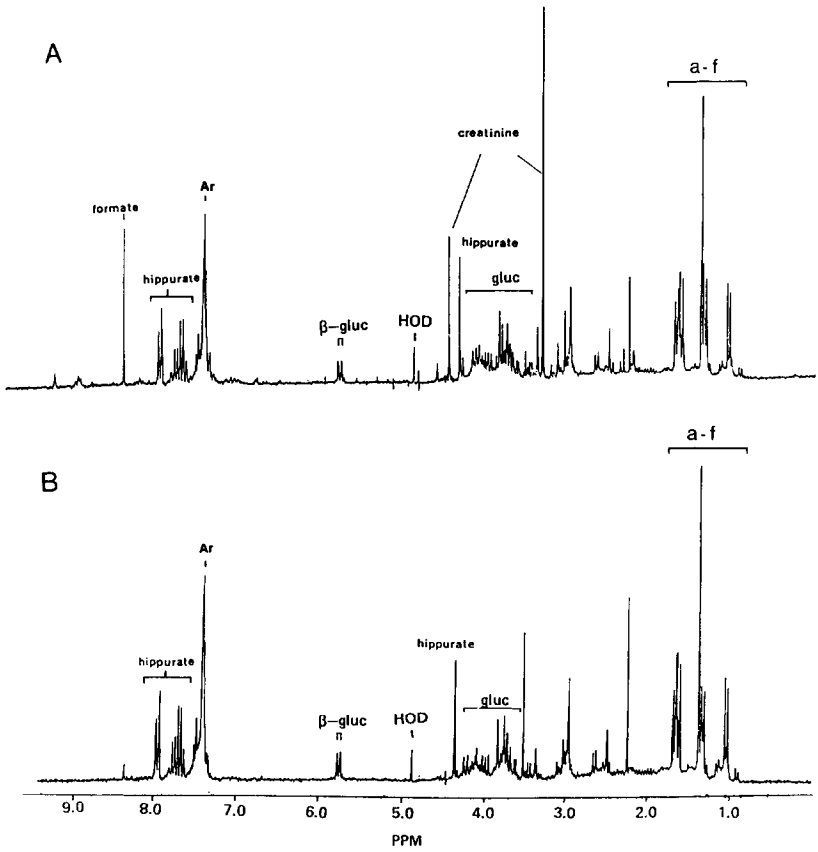

Figure 3
^{1}H NMR spectra obtained from column eluates following the application of 2 ml of the acidified ibuprofen metabolite containing urine shown in Fig. 2 to either 100 mg of carbopack (A) or 100 mg of C_{18} bonded silica gel (B). The adsorbed material present on the carbopack was eluted with chloroform/methanol (50:50, v/v) and the C_{18} packing with methanol. Samples were redissolved in D_2O for spectroscopy.

the latter. Using the acidic conditions employed here (necessary to preserve the unstable glucuronide conjugates intact) creatinine is not retained at all on the C_{18} phase. The extraction of creatinine by the Carbopack material was not complete, with considerable amounts detectable in the non-retained portion of the sample. Nevertheless, the extraction of creatinine at all suggests that carbon may be a relatively stronger adsorbent than the C_{18} bonded phase.

However, despite a greater apparent affinity of Carbopack for organic solutes it was found that in experiments where increasing amounts of urine were loaded onto a 100 mg of each phase that the C_{18} bonded silica gel had a greater capacity than the carbon based material for the ibuprofen metabolites.

Because of the pH dependent stability of the ester glucuronide metabolites of ibuprofen a detailed study of the effects of pH on extraction onto Carbopack was not undertaken. However, when a freshly voided urine sample (pH 6.8) was applied to a Carbopack cartridge, differences in the spectra of the retained components, relative to that obtained for the same sample applied after acidification, were noted. Thus, in the

pH 6.8 sample, ibuprofen glucuronide (the least "polar" of the three metabolites) was well retained, but the signals for both HMPPP and CMPPP were attenuated compared with those present in the spectrum of the acidified aliquot. The use of acidified methanol and water in the conditioning of the cartridge seems less critical than acidification of the sample, with high extraction of the ibuprofen metabolites obtained whether or not formic acid was present in the prewash solvents.

In the case of the ibuprofen metabolites a study of the effects of the composition of the eluting solvent on the recovery of material from the cartridges was undertaken (previously similar studies for such samples on C_{18} Bond Elut^{TM} columns have been reported [6–8]). The effect of variations in solvent composition are illustrated in Fig. 4. As found for the β-blockers pure methanol and chloroform were poor eluents. Clearly, not only was the total recovery strongly dependent on the solvent composition but the nature of the substances eluted also varies.

Thus, with pure chloroform as eluent only the most "polar" metabolites were eluted, whilst IG remained largely on the packing (thus allowing selective enrichment of this metabolite if required). Pure methanol eluted all the metabolites, albeit with relatively poor recoveries. The best results were obtained with a chloroform/methanol ratio of 40:60 (v/v). Complete recovery of adsorbed material was also possible using sequential washing with pure chloroform followed by methanol (or indeed methanol then chloroform). However, this is presumably due, at least partly, to the combined eluotropic strength of the residual solvent remaining in the cartridge combining with the new eluent to give a situation more akin to that observed with the mixtures. Attempted elution with methanol–water based mixtures was, predictably, not successful.

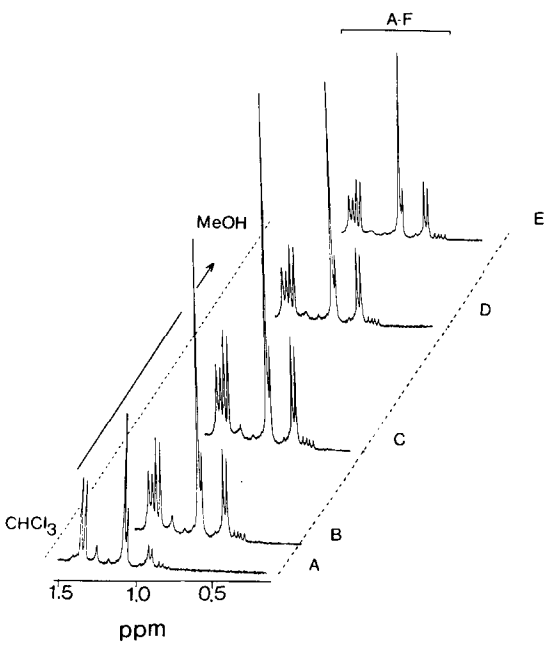

Figure 4
Partial ^{1}H NMR spectra showing the resonances of the methyl groups of the ibuprofen metabolites present in the 2–4 h post-dose urine sample (see Fig. 2) following application of 2 ml samples to 100 mg carbopack and recovery with chloroform–methanol eluates of varying composition. Key: A, 100% chloroform; B, chloroform/methanol, 60:40; C, chloroform/methanol, 40:60; D, chloroform/methanol, 20:80; E, 100% methanol.

Conclusions

The introduction of SPE has proved to be of considerable value in the analysis of drugs and their metabolites present in trace amounts in biological fluids. Continuing innovation in this area, including the introduction of new types of solid phase will further extend the application of SPE based methods. As yet it is not possible to predict how materials such as graphitized carbon black will fit into the pre-existing, largely silica based, phases. However, based on the results reported here and elsewhere [1–3] it is clear that the carbon has different properties to C_{18} bonded silica gel and is capable of performing efficient extraction of acidic and basic drugs from plasma and urine. In some circumstances the absence of silanols on the phase may also be advantageous. The requirement for elution with volatile organic solvents, rather than the aqueous–organic solvents generally employed for the C_{18} bonded materials, may also prove to be of benefit if there is a requirement to concentrate the sample further prior to analysis, or if a subsequent normal-phase HPLC or GC separation is contemplated.

Additional studies to further characterize the properties of graphitized carbon black as an SPE material, and answer some of the questions raised by these preliminary studies are continuing.

References

[1] C. Borra, A. Di Corcia, M. Marchetti and R. Samperi, *Analyt. Chem.* **58**, 2048–2052 (1986).
[2] K. R. Kim, Y. J. Lee, H. S. Lee and A. Zlatkis, *J. Chromat.* **400**, 285–291 (1987).
[3] B. Barfagnini, A. Di Corcia, M. Marchetti and R. Samperi, *Chromatographia* **23**, 835–838 (1987).
[4] R. J. Ruane and I. D. Wilson, *J. Pharm. Biomed. Anal.* **5**, 723–727 (1987).
[5] 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. 295–300. Plenum Press, New York (1988).
[6] I. D. Wilson and I. M. Ishmail, *J. Pharm. Biomed. Anal.* **4**, 663–665 (1986).
[7] I. D. Wilson and J. K. Nicholson, *Analyt. Chem.* **59**, 2830–2832 (1987).
[8] I. D. Wilson and J. K. Nicholson, *J. Pharm. Biomed. Anal.* **6**, 151–165 (1988).

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