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Comparison of the properties of polymeric and C8 based materials for solid phase extraction

Paul Martin *, Ian D. Wilson

Department of Safety of Medicines, Zeneca Pharmaceuacals, Mereside, Alderley Park, Macclesfield, Cheshire SK10 4TG, UK

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Abstract

The extraction properties of two polymeric solid phase extraction materials, styryldivinyl benzene (SDB) and 'Oasis'™ have been compared with those of a base deactivated C8 bonded silica gel using a range of acidic and basic test analytes. In the case of the two polymer phases good extraction of all the test compounds from aqueous buffer was obtained over the pH range 2–10. On the C8 material, efficient extraction of the most polar acidic analyte, anisic acid, was only obtained between pH 2 and 6. The use of methanol water mixtures, or methanol–water mixtures modified with either trifluoroacetic acid (TFA) or triethylamine (TEA) as eluents was investigated for the recovery of the analytes following extraction. The use of TFA or TEA as ionic modifiers strongly influenced the efficiency of the elution step. The effect of a plasma matrix on extraction efficiency was also investigated, with the result depending upon the analyte. An approach to assessing the performance of the three phases has been developed based on the percentages of methanol in the eluent resulting in the recovery of 50% of the analyte, and in determining the difference between eluents giving recoveries of 10 and 90%.

Keywords: Styryldivinylbenzene; 'OASIS'; C8 bonded silica; Anisic acid; Propranolol; ICI 128,436

1. Introduction.

Sample preparation remains a central feature of pharmaceutical and biomedical analysis, and the time consuming nature of the current methodology has resulted in a continuous search for new and improved techniques. Solid phase extraction (SPE) is an important area of innovation for biomedical sample preparation, with the steady introduction of new phases, or formats and the development of fully automated methods. The bulk of the commercially available phases are based on bonded silicas of one type or another (e.g. C18, C8, C2 etc.). Whilst these have been very successful the presence of residual silanols has often complicated the extraction of basic compounds because of ionic interactions e.g. [1–4]. Recently a number of non-silica based polymeric SPE sorbents have been introduced which offer the potential for a different range of selectivities to silica based materials. This study was performed to assess the extraction behaviour of a range of compounds on two polymeric SPE phases, based on either (poly[divinyl-co-*N*-

* Corresponding author.

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vinylpyrrolidinone]) (OASIS™) and styryldivinylbenzene, and compare these materials with a base deactivated C8 bonded silica gel. Extraction properties were determined for model acidic and basic analytes in order to investigate the relative merits of each of the 3 phases and to see what advantages, if any, the polymeric materials offer over 'conventional' silica-based SPE phases of the type investigated in our previous studies.

2. Experimental methods

2.1. Chemicals

Methanol and triethylamine (TEA) were purchased from Fisons (Loughborough, UK). The buffer solutions used for extractions were 0.1 M sodium citrate buffer (pH 2–4) and 0.2 M sodium acetate buffer (pH 5–10). The buffers were adjusted to the appropriate pH using 0.1 M sodium hydroxide or acetic acid.

2.2. Cartridges

The cartridges used in this study were the 30 mg OASIS™ (Waters, Milford, Massachusetts), 50 mg styryl divinylbenzene (J.T. Baker Phillipsburg, New Jersey) and 100 mg RP Select B™ C8 bonded silica (Merck, Darmstadt, Germany). The cartridges were used as supplied.

2.3. Test compounds

Three radiolabelled test compounds (for structures see insets to Figs. 1–3) were employed in these studies. [^{14}C]-propranolol [2(*R*,*S*)-1-(1-amino-1-methylethyl-3-(naphthyloxy)propan-2-ol], (specific activity 38.4 μCi mg^{-1}) and ICI 128,436 {2-(4-bromo-2-fluoro-benzyl)-1,2-dihydro-1-oxo-[1-4]-4-phthalazinylacetic acid}, (specific activity 24.4 μCi mg^{-1})were synthesised in the radiochemical laboratory, Zeneca Pharmaceuticals, and had a radiochemical purity $>95\%$ (by TLC). [^{14}C]-Anisic acid (specific activity 18 μCi mg^{-1}) was purchased from Amersham International, and also had a radiochemical purity $>95\%$.

2.4. Solid phase extraction and elution

2.4.1. Effect of pH on extraction

To examine the effect of the pH of the sample on the extraction of each of the test compounds the phases were conditioned with methanol (0.5 ml), water (0.5 ml) and buffer (0.5 ml) at the pH to be used for extraction. The samples containing the test compounds (1.0 ml, 2.5 μg ml^{-1}) were then applied at pH 2, 4, 6, 8 or 10. The cartridge was then washed with buffer at the same pH. The eluents were collected into scintillation vials and analysed by liquid scintillation counting in order to assess recovery.

2.4.2. Cumulative elution experiments

The elution of the test analytes from each of the three SPE phases was determined following extraction from aqueous buffer (pH 2 anisic acid

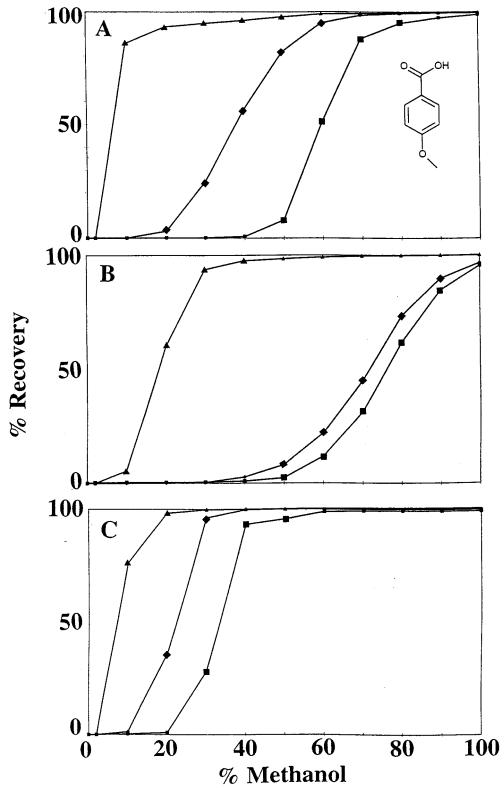

Fig. 1. Cumulative elution curves for anisic acid from (A) OASIS (B) SDB and (C) C8. ◆ = methanol: water, ▲ = methanol: water: TEA, ■ = methanol: water: TFA.

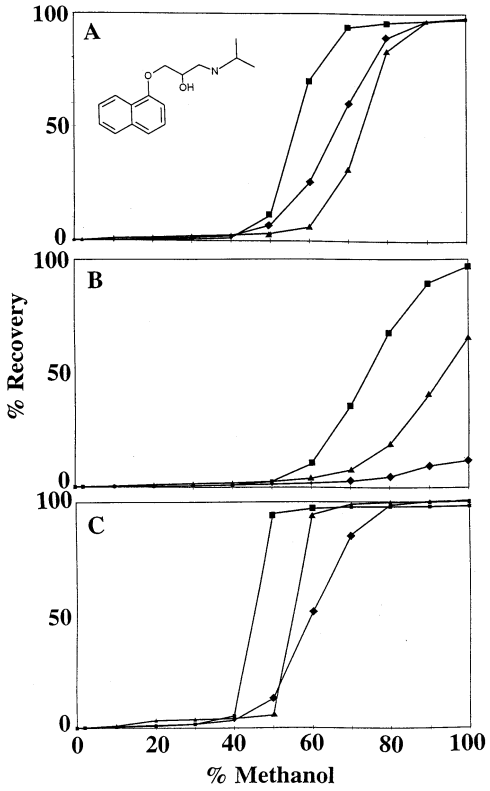

Fig. 2. Cumulative elution curves for propranolol from (A) OASIS (B) SDB and (C) C8. Symbols as for Fig. 1.

and ICI 128,436, pH 5 for propranolol). Cartridges were first conditioned by washing sequentially with methanol (0.5 ml), water (0.5 ml) and finally with a buffer (0.5 ml) at the same pH as the sample. After conditioning, an aliquot (1 ml) of the sample in buffer, containing 2.5 μg of [^{14}C]-radiolabeled compound was applied to the cartridge followed by a 1 ml wash with buffer. All of the eluates from the column were collected separately and analysed by liquid scintillation counting. Cartridges were eluted with methanol: water mixtures of increasing eluotropic strength (10, 20, 30, 40, 50, 60, 70, 80, 90 and 100% methanol). This elution protocol was performed with methanol–water and also with methanol–water modified with either 1% TFA or TEA (methanol–TFA and methanol–TEA).

2.5. Effect of plasma on extraction efficiency

In order to investigate the effect of plasma proteins on extraction efficiency samples (1.0 ml) containing 2.5 μg of each of the analytes and 25% dog plasma were extracted on to each of the three types of SPE cartridge at pH 2 and 10 using the conditions described above. Radioactivity in the eluates was determined using scintillation counting.

2.6. Liquid scintillation counting

Liquid scintillation counting was performed by mixing the eluates from the columns with 10 ml of scintillation fluid (ready value scintillation fluid, Beckman) in 20 ml glass scintillation vials. Samples were analysed on either a Packard TRI CARB 1900 CA or Beckman LS 1801 liquid scintillation counter with quench correction.

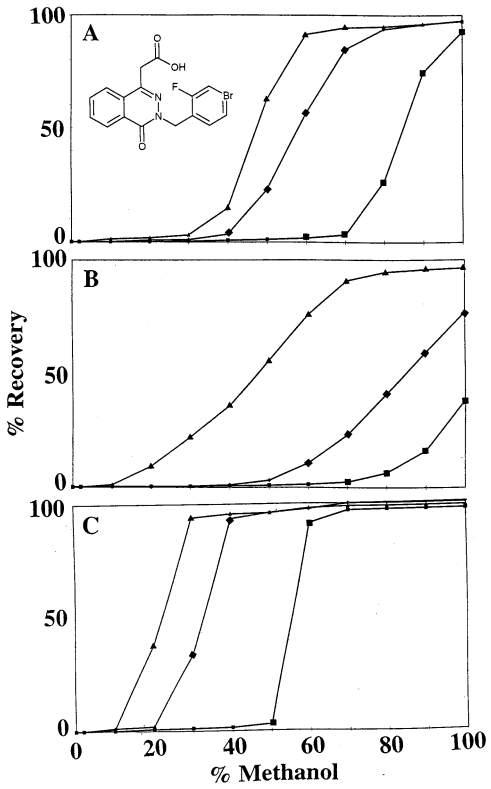

Fig. 3. Cumulative elution curves for ICI 128,436 from (A) OASIS (B) SDB and (C) C8. Symbols as for Fig. 1.

3. Results and discussion

3.1. Anisic acid

Anisic acid (*p*-methoxybenzoic acid, see inset to Fig. 1 for structure) is a polar aromatic acid which is relatively poorly retained on conventional bonded phases and is typical of the many acidic analytes which can pose difficulties in sample preparation.

The analyte was completely retained on OASIS™ and SDB when extracted from buffer over the pH range from 2 to 10. On the RPB phase, complete retention was achieved at pH between 2 and 6. Thereafter, extraction efficiencies rapidly decreased as the pH was increased to pH 8 (76%) and pH 10 (60%). The poor retention on the C8 phase clearly only occurred when the compound was primarily in the ionised form. Compared to the C8 material therefore the OASIS and SDB materials provided a much wider range of options for varying extraction pH to ensure complete retention of this analyte. In contrast, on the C8 material the extraction pH is clearly more critical, and needs to be maintained within a limited range in order to achieve high extraction efficiencies. However, the results obtained show that all 3 phases could be used to extract anisic acid efficiently.

Matrix effects can be a source of difficulty in sample preparation, and in particular protein binding effects can cause problems for acidic analytes (which are usually strongly protein bound). However, the presence of dog plasma had little effect on extraction efficiency on the OASIS cartridges, with 99% retained at pH 2 and 97% at pH 10. In the case of the SDB phase the effects of plasma at pH 2 was negligible, with over 99% extracted. However, at pH 10 some 21.5% of the anisic acid passed through the cartridge unretained. Similarly, whilst the RPB material effectively extracted over 99% of the analyte at pH 2, the presence of dog plasma markedly reduced extraction efficiency to only 28% at pH 10. Such a result would be typical of the extraction of a highly protein bound acidic analyte on a silica-bonded phase.

As extraction of anisic acid from buffer at pH 2 resulted in complete retention on all three types of cartridge this pH was used for further studies. The recovery of the adsorbed material was then investigated by stepwise gradient elution using methanol–water mixtures (either alone or modified with TFA or TEA). The cumulative elution profiles for anisic acid from OASIS, SDB and RPB Select B are presented in Fig. 1A, B and C respectively). On each of the three phases the order of the relative eluotropic strengths of the methanol: water, methanol: water: TFA and methanol: water: TEA mixtures was the same. Thus, methanol containing TEA was the most effective elution solvent (required the smallest proportion of methanol to achieve recovery). Methanol: water was next best and methanol–TFA the least effective. This order of elution can be explained by the elution solvent pH and the consequent ionisation of the compound. At pH 2, in the presence of TFA, anisic acid was maintained in its unionised form and was well retained by a reversed-phase mechanism on all three sorbents. At pH 8 in the presence of TEA, the compound was ionised, more polar and not well retained by partition. The methanol: water solvent systems, which were essentially neutral, provided conditions, and thus retention behaviour, intermediate between the TEA and TFA-modified systems.

The position and shape of the elution curves across the phases shows difference between the various sorbents. Comparing the methanol–TFA curve for each phase it was evident that the C8 phase was the least retentive followed by OASIS and SDB. The percentage methanol required to recover 50% of the anisic acid from each phase was $\sim$60% (OASIS), 33% (C8) and 75% (SDB). Elution curves for the OASIS and C8 phases were relatively sharp, indicating good mass transfer properties and the likelihood that the analyte could be recovered in a small volume of solvent. In comparison the elution curve for the SDB material was rather shallow, probably due to poor mass transfer of the compound from the phase into the surrounding eluent. The consequence of the poor mass transfer is that large elution volumes are necessary to achieve elution and it is

also likely that extractions will be less selective. Sharp elution profiles were possible from the SDB material with TEA as a modifier, giving 50% recovery, estimated from the curves shown in Fig. 2 of ca. 17% methanol (ca. 5% for OASIS and C8). Essentially complete recoveries were seen with methanol contents of 20% for C8 and OASIS and 30% for SDB.

3.2. Propranolol

Propranolol (structure in inset to Fig. 2) was extracted onto each phase across the pH range 2–10. Greater than 99% of the applied compound was retained on all 3 phases across the complete pH range. These results indicate that all three phases could be used to extract the compound with good efficiency from buffer. However, this extraction protocol was not useful in demonstrating differences between the phases. The effect of a plasma matrix was therefore examined. For the OASIS and C8 materials the presence of dog plasma in the samples had little effect, with ca. 99 and 96% extraction efficiencies at pH 2 and 10 respectively. For the SDB polymer 99% extraction was seen at pH 2, but this fell to only 86% at pH 10.

Having determined the effects of pH on the extraction of propranolol the cumulative elution curves were obtained for each of the phases. These results are presented in Fig. 2(A–C). At an application pH of 5, propranolol was completely retained on all 3 phases.

When elution with methanol water etc. from the C8 material was performed all three elution curves were steep with methanol–TFA proving to be the strongest eluent for propranolol on this phase with complete recovery obtained with ca. 50% methanol. Methanol–TEA was slightly more eluotropic than methanol–water. Thus the bulk of the analyte was eluted with 60% methanol–TEA whilst 80% methanol–water was required to achieve the same result. Previous experiments [1–4] with conventional, non-base deactivated, ODS phases gave negligible recovery with methanol–water eluents due to strong silanophilic interactions. The base deactivated C8 enabled methanol–water to be an effective eluent indicating greatly reduced silanophilic interactions. However, it is possible that the slight superiority of the TEA-containing eluents noted above is due to some residual silanol activity.

Compared to the C8 material the OASIS SPE phase was much more retentive for propranolol with negligible recovery of the analyte obtained with 40% methanol (with or without TFA or TEA). Methanol–TFA was the strongest eluent achieving 70% recovery with 60% methanol. Methanol–TEA was the least eluotropic solvent whilst methanol–water mixtures were intermediate between methanol-TEA and methanol–TFA mixtures. However, as shown in Fig. 2(A) all 3 elution curves were quite close together, and showed greater retention than the C8 bonded phase. Propranolol was most easily recovered in eluents containing TFA and this can be explained by assuming that the acid environment favours ionisation of the analyte reducing the compounds affinity for the reversed-phase sorbent. This view is supported by the relative positions of the other two curves. The basic pH of the TEA-containing eluent ensured that the analyte was present in the unionised state ensuring efficient retention on the phase.

As seen on the OASIS and C8, propranolol was well retained on SDB at pH 5. Negligible recovery was achieved with proportions of methanol of up to 50% even in the presence of TFA or TEA. However, as the methanol content was increased further greater recovery was achieved. The resulting elution curves (Fig. 2C) were very shallow, irrespective of the composition of the elution solvent compared to curves on the other two phases. Methanol–TFA-based eluents were the most effective, and complete recovery could be achieved with 100% methanol–TFA. Methanol–TEA solvents were the next most efficient, though only 70% recovery was achieved with 100% methanol–TEA. It was not clear why methanol–TEA should be better than methanol–water. In basic conditions propranolol would be unionised and this should favour retention. The behaviour could be explained if propranolol was retained by some secondary mechanism in addition to simple reversed-phase partition (e.g. either ionic or hydrogen bonding). If such interactions occurred, the

TEA might compete with propranolol for these interactions with the phase and thus displace the compound. Methanol–water was a very poor eluent for the elution of propranolol from SDB, which does imply a more complex retention mechanism on this phase than reversed-phase interactions alone.

3.3. ICI 128,436

Like anisic acid, ICI 128,436 (structure in inset to Fig. 3) is an acidic compound, but is somewhat less polar, and consequently more easily extracted. This analyte was also applied to each of the three phases across the pH range 2–10. Similarly to propranolol, and in contrast to anisic acid, $>99\%$ of the applied compound was retained from aqueous buffer, on all three phases, across the entire pH range. However, the effect of dog plasma was to reduce extraction efficiency on all three phases. The effects were most marked on the SDB material, with losses of 9 and 24% on application at pH 10 and 2 respectively. Losses on the Oasis and RPB cartridges were comparable at ca. 3% for pH 2 and 12% for pH 10.

Cumulative elution profiles for ICI 128,436 were then obtained for each of the 3 phases, as illustrated in Fig. 3(A–C). The order of the elution curves on each phase was the same for all 3 materials with the eluotropic strength decreasing in the order methanol–TEA, methanol–water and methanol–TFA. As with the anisic acid, these trends can be rationalised in terms of the ionisation state of the compound in eluents of different pH. With basic eluents (i.e. methanol–TEA) the carboxylic acid moiety was ionised, more polar and the analyte was therefore less well retained via reversed-phase interactions. At the other extreme of pH, under acidic conditions (i.e. methanol–TFA the compound was presumably in its unionised form and thus less polar and more strongly retained.

Although the order of the elution curves was the same on each phase there were notable differences in the elution profiles across phases. The elution curves were relatively steep on the OASIS and C8 phases irrespective of the elution solvent used. In contrast, the elution curves for all solvents were relatively shallow on the SDB material which was again indicative of poor mass transfer on this phase.

Comparing the elution curves for the weakest eluent (methanol–TFA) marked differences were noted between the phases with the least retention of the ICI 128,436 on the C8 phase, greater retention on Oasis, and incomplete recovery (only ca. 35%) on SDB.

4. Comparison of phases

Clearly the data presented above show a range of different properties for these SPE materials, and it would be valuable to have an objective means of comparing between them. Visual inspection of the cumulative elution curves is quite revealing, yielding information on both strength of retention and ease of elution. Indeed from an examination of this type of data it is possible to define the properties of the 'perfect' SPE material. Thus, a perfect phase might be considered to be one which retains analytes well with little possibility of loss on application, but from which the analyte can be quantitatively eluted in a narrow band of eluent compositions. This would result in relatively steep elution curves, with the solvent giving complete elution as close as possible to that which resulted in complete retention. In addition, in order to be useful, the phase needs to be able to discriminate between analytes with different physicochemical properties, otherwise it would concentrate everything in the sample and show no selectivity.

One objective method of assessing performance and making comparisons would be to examine the methanol concentrations that resulted in the recovery of 10, 50 and 90% of the analyte. These values would then equate to the 'elution solvent' (ES) 10, 50 and 90. Comparison of ES_{50} values between phases would give a rapid comparison of how well retained an analyte was on each phase. Thus the higher the ES_{50} the more retained the analyte is retained on a particular phase. The ES_{10} value gives a limit on the solvents that can be used to wash the cartridge following sample application, and the ES_{90} provides the composition of

Table 1
ES_{50} and ES_{90-10} values for anisic acid, propranolol and ICI 128,436 on OASIS, SDB and C8 phases

Anisic acid	OASIS ES_{50}	OASIS ES_{90-10}	SDB ES_{50}	SDB_{90-10}	C8 ES_{50}	C8 ES_{90-10}
Methanol/TFA	60	23	76	37	33	16
Methanol/water	38	33	72	40	22	16
Methanol/TEA	6	15	18	18	7	14
Propranolol						
Methanol/TFA	57	19	74	31	45	8
Methanol/water	67	29	>100	NC	60	26
Methanol/TEA	74	23	94	NC	55	9
ICI 128,436						
Methanol/TFA	85	26	>100	NC	55	9
Methanol/water	58	33	85	NC	33	16
Methanol/TEA	47	25	47	48	22	17

NC, not calculable.

a suitable eluent for analyte recovery. In addition, the difference in methanol concentration between the ES_{10} and ES_{90} values (ES_{90-10}) is essentially another way of estimating the slope of the elution curve. Thus high values of ES_{90-10} describe shallow elution curves and poor mass transfer. Such high values would result in the need for large elution volumes and less selective extractions.

The ES_{50} values for all three analytes on the C8, OASIS and SDB cartridges, for all three elution solvents are given in see Table 1. Comparison of the ES_{50} for any of the three test compounds reveals differences between phases and elution solvents. For example, the propranolol ES_{50} values show a clear trend where, for any elution solvent this increases in the order RPB < OASIS < SDB. A similar trend was observed for ICI 128,436 and anisic acid in the majority of cases. Comparing between analytes, where large differences in the ES_{50} would indicate the selectivity of the phase it is evident that the use of methanol–TEA maximises the differences in extraction properties on all three types of cartridge. For example on the OASIS ES_{50}s', of 6, 47 and 74 can be estimated for anisic acid, ICI 128,436 and propranolol respectively, whilst with methanol–TFA the equivalent values are 60, 57 and 85.

Comparing the ES_{90-10} values for anisic acid on the different phases it was evident that there was considerable variation in the result (14–40%) with the lowest values (14–16%) obtained on the RPB phase irrespective of elution solvent. This result indicates steep elution curves, as shown in the figures. The SDB phase in contrast had ES_{90-10} values ranging from 18–40%. Thus using methanol–TEA ($ES_{90-10} = 18\%$) recovery was efficient, characterised by a sharp elution curve. At the opposite extreme, using methanol–TFA as eluant the ES_{90-10} was twice that for methanol–TEA (37%) indicating less efficient recovery from the phase. It was noticeable that the higher ES_{90-10} values were associated with higher ES_{50} values. On the OASIS phase, there was also a range of ES_{90-10} values from 15 (methanol–TEA) to 33% (methanol–water). Clearly, it would generally be better to select combinations of phases and eluting solvents with the smallest possible ES_{90-10} values.

5. Conclusions

All three phases proved to be capable of extracting the test analytes efficiently from buffer solutions at a range of pH. The polymeric phases differed from the C8 material in being able to efficiently extract anisic from aqueous buffer over the whole of the pH range studied, whilst the silica-based material showed a marked degree of pH dependence for this particular analyte. Matrix effects were observed, but plasma had the least effect on extraction onto the OASIS cartridges.

In general the C8 and OASIS materials showed sharper elution curves than the SDB material, which would probably allow for recovery of analytes in smaller volumes. The sharp elution profiles also suggest that somewhat more selective extractions might be possible on these phases.

The polymeric materials generally demonstrated superior retention of the test analytes from aqueous buffer solutions compared to the silica-based C8 phase, particularly for highly polar analytes such as anisic acid. However, differences in performance between the OASIS and SDB materials were observed in the presence of a plasma matrix.

Polymers such as SDB and OASIS may well provide useful alternatives to 'traditional' silica based phases for SPE. However, whilst giving good extraction efficiencies the poor elution profiles of the SDB material may prove to be a disadvantage under some circumstances.

References

[1] R.J. Ruane, I.D. Wilson, J. Pharm. Biomed. Anal. 5 (1987) 723.
[2] D.W. Roberts, R.J. Ruane, I.D. Wilson, J. Pharm. Biomed. Anal. 7 (1989) 1077.
[3] P. Martin, E.D. Morgan, I.D. Wilson, J. Pharm. Biomed. Anal. 14 (1996) 419.
[4] P. Martin, E.D. Morgan, I.D. Wilson, Anal. Chem. 69 (1997) 2975.