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Short Communication

Selective solid-phase extraction of basic drugs by C_{18}-silica. Discussion of possible interactions

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Introduction

The solid-phase extraction of drugs from body fluids using C_{18}-silica usually involves four steps, namely:

(i) conditioning of the C_{18}-silica sorbent, by washing with a water miscible organic solvent (preferably methanol) and then with water;
(ii) application of the biological sample;
(iii) washing the sorbent with water or another suitable liquid to remove trapped endogenous compounds;
(iv) elution of the drugs with an appropriate solvent.

Of these four steps, the third could be the crucial one since by efficient washing of the C_{18}-silica sorbent to remove the endogenous compounds a significant amount of a given drug may be lost.

The different abilities of organic solvents to elute drugs from octadecylsilyl silica may serve as one of the approaches for solving the problem. This difference, mainly in the case of the two water miscible solvents, methanol and acetonitrile, was observed and subsequently utilized in the Author's laboratory for the selective solid-phase extraction of various basic drugs. These included, alkoxycarbanilate local anaesthetics carbisocaine, heptacaine and pentacaine [1–5], the antiarrhythmic mexiletine [6], the cardioprotective drug stobadin [7], and several beta-blockers [8]. The technique uses to advantage the low elution strength of acetonitrile to release basic drugs from C_{18}-silica. The procedure of selective solid-phase extraction involves five steps, namely:

(i) conditioning the sorbent with methanol and water;
(ii) application of the biological sample;
(iii) washing the sorbent with water to flush-out hydrophilic endogens;
(iv) washing the sorbent with acetonitrile to remove the majority of hydrophobic endogens;
(v) elution of the drugs, still retained on the sorbent, with methanol.

Sorbents such as, Sep–Pak C18 [1], Silipore C18 [2, 3], CP–Elut C18 [4] and Separcol SI C18 [5–8] may be used with the technique.

The advantage of the lower elution strength of acetonitrile to release basic drugs from C_{18}-silica was recently utilized by Harrison *et al.* for increasing selectivity of the solid-phase extraction procedure of propranolol on Bond-Elut C18 cartridges [9] and by Ruane and Wilson for solid-phase extraction of several beta-blockers using with Bond-Elut C18 and Baker C18 cartridges [10]. Ruane and Wilson also discussed the possible rôle of free silanol groups of the C_{18}-silica sorbents to explain the unexpected elution behaviour of beta-blockers when acetonitrile was used as the drug eluting liquid.

The aim of the present work was to resolve the problem of different elution strengths of

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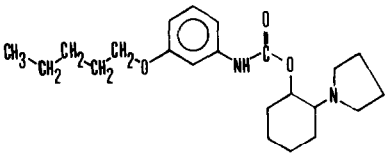
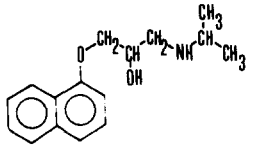
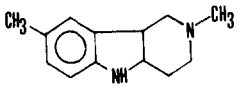
methanol and acetonitrile towards basic drugs, taking into account mainly properties of the support. The basic drugs used in the study were pentacaine (pK_a = 8.6 [11]), propranolol (pK_a = 9.45 [12]) and stobadin (pK_a = 8.71 [13]).



PENTACAINE



PROPRANOLOL



STOBADIN

Experimental

Materials

Propranolol and the internal standard oxprenolol were obtained commercially. Pentacaine was kindly supplied by Galena (Opava, Czechoslovakia) and the corresponding internal standard, the *O*-hexyl analogue, was synthesized at the Faculty of Pharmacy, Comenius University (Bratislava, Czechoslovakia). Stobadin and its internal standard, the *N*-ethyl analogue, was prepared at the Institute of Organic Chemistry and Biochemistry (Prague, Czechoslovakia).

Octadecylsilanized silica was prepared according to the method of Buszewski *et al.* [14] using the 40–100 μm fraction of silica gel Silpearl (surface area 610 $m^2 g^{-1}$, pore volume 0.65 ml g^{-1}) supplied by Kavalier Works (Votice, Czechoslovakia), using a mixture of C_{16}–C_{18}-dimethylmonochlorosilanes of C_{16}–C_{18} ratio 1:2. End-capping was performed by means of reaction with hexamethyldisilazane according to the method of Unger [15]. The carbon content of C_{18}-silica material was 22.7% m/m, the end-capped product contained 23.3% of carbon.

The individual drugs were dissolved and subsequently extracted from, three 5-mM buffers, namely, acetate (pH 4.4), Tris (pH 7.4) and carbonate buffer (pH 9.4).

Analyses

Capillary gas–liquid chromatography was used for the determination of the recovery of the drugs eluted from the cartridges. A Hewlett–Packard Model 5880A gas chromatograph was used in conjunction with a 5880A level four terminal and a Model 7673A Hewlett–Packard autosampler. The chromatograph was equipped with a thermionic nitrogen detector (NPD). A wide-bore fused silica column HP-1 (30 m, 0.53 mm, i.d., film thickness 0.88 μm) Hewlett–Packard (Wien, Austria) was used. The temperature of the direct injection port and of the detector was 300°C, that of the column 220, 185 and 145°C for the determination of pentacaine, propranolol and stobadin, respectively. Nitrogen used as a carrier gas was maintained at a flow rate of 25 ml min^{-1}; no auxiliary gas was used. Purge activation time was 30 s. Pentacaine and its internal standard were methylated before analysis [5].

Extraction procedure

The in-house extraction columns (100 mg of sorbent) were conditioned before use by washing with 2 ml of methanol and 1 ml of water prior to the addition of 1 ml of the sample solution (1 μg of pentacaine, propranolol or stobadin in the appropriate buffer). After passage of the sample solution through the cartridge 1 ml of water was applied. The residual water was removed by a slight flow of nitrogen. Three 1-ml portions followed by one 2-ml portion of the eluting solvent, either acetonitrile or methanol, were then applied. In the case of the displacement of stobadin by methanol, a 3-ml portion was eventually used. Individual portions of eluate were collected into 3-ml cone vials Reacti-Vials, Pierce (Oud-Beijerland, The Netherlands) containing 1 μg of the appropriate internal standard. The solvent was evaporated to dryness at 50°C under nitrogen. To the dry residue 250 μl of ethyl acetate was added, the vial was stoppered and agitated on a Vortex mixer for 10 s. Three microlitres of this solution were injected into the gas chromatograph by means of the autosampler. In the case of the pentacaine GLC analysis, 10 μl of the trimethylanilinium hydroxide solution (0.1 M in methanol; Serva, Heidelberg, FRG) was added to the autosampler vial to perform on-column drug derivatization [5]. The drug–internal standard peak area ratios were compared with those

obtained after mixing 1 μg of the drug with 1 μg of the internal standard, and cumulative curves were used to form the elution profiles. All evaluations were carried out in triplicate.

Results and Discussion

The most significant fact revealed by the recovery profiles shown in Figs 1–3 is the different elution ability of methanol and acetonitrile towards the drugs tested. The difference is observable regardless the drug, support and concentration of hydrogen ions in the extracted solution.

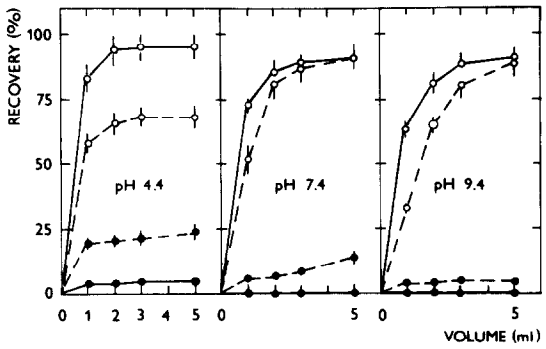


Figure 1
Elution profiles of propranolol at different pH using C_{18}-silica sorbent (——) and end-capped C_{18}-silica sorbent (----) when washed with methanol (○) and acetonitrile (●).

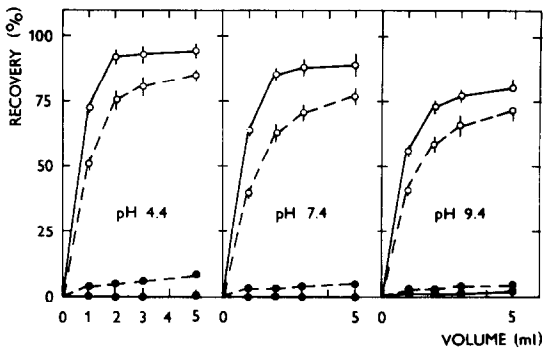


Figure 2
Elution profiles of pentacaine at different pH using C_{18}-silica sorbent (——) and end-capped C_{18}-silica sorbent (----) when washed with methanol (○) and acetonitrile (●).

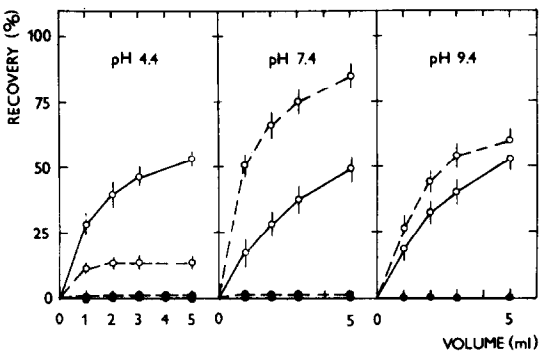


Figure 3
Elution profiles of stobadin at different pH using C_{18}-silica sorbent (——) and end-capped C_{18}-silica sorbent (----) when washed with methanol (○) and acetonitrile (●).

Recovery with methanol

As shown in Figs 1–3, 5 ml of methanol was sufficient for the elution of pentacaine and propranolol, however, for stobadin 8 ml of the eluent was required. The individual recoveries are summarized in Table 1. The manufacturer-to-manufacturer and batch-to-batch variations previously reported for adsorbents [16, 17] were again observed in the present study. Recoveries in previous experiments with selective solid-phase extraction were 100% for pentacaine and propranolol (unpublished results) and 84% for stobadin [7], when extracted from water.

On comparing the pH dependence of the recoveries of the drugs practically no difference was observable using normal C_{18}-cartridges. Only in the case of pentacaine was a slight increase of recovery with decreasing pH, in the range studied, recorded. Entirely different results, however, were obtained with

Table 1
Recovery of pentacaine, propranolol and stobadin expressed as a percentage of the total amount applied to the C_{18}-cartridges (A) and end-capped C_{18}-cartridges (B) when washed with methanol

	Recoveries (%)*								
	pH 4.4			pH 7.4			pH 9.4		
Drug	A	B	A–B	A	B	A–B	A	B	A–B
Pentacaine	94 ± 3	82 ± 2	9	89 ± 4	77 ± 3	12	80 ± 4	72 ± 5	8
Propranolol	95 ± 6	68 ± 4	27	90 ± 4	91 ± 6	−1	90 ± 4	89 ± 5	1
Stobadin	62 ± 3	13 ± 2	49	60 ± 7	92 ± 8	−32	70 ± 6	70 ± 7	0

*Mean ±SD, n = 3.

end-capped C_{18}-silica, when pH dependences of recoveries were observed depending upon the nature of the drug studied. Pentacaine recoveries were lower with end-capped material, but these differences were pH-independent, achieving in all three cases the value of about 10%. The behaviour of propranolol corresponded better to its ionization (pK_a = 9.45 [12]), as the recovery decreased by about one-third at the lowest pH. In the case of stobadin, another type of pH dependence was obtained, showing an expected decrease of recovery at the lowest pH, yet an unexpected increase at pH 7.4.

In this connection the question arose concerning the mechanism of adsorption of basic drugs on the C_{18}-silica studied. Recent examinations employing small probe molecules indicated that non-polar solutes appeared to have the most favourable interaction near the centre of the solvated chains, whereas polar basic solutes penetrated the layer deeply to interact with acidic sites of the silica surface [18]. The present findings are in agreement with this mechanism. At high pH, the molecules of basic drugs are unionized, interacting only with alkyl chains of the C_{18}-sorbent. In the acidic medium, ionized and thus polar molecules penetrated deeply, with their polar "heads" downwards. A change in the content of free silanol groups affected their extraction more markedly than that of non-polar drugs from basic solutions. In Figs 1–3, there are greater differences between elution profiles for the C_{18}- and end-capped C_{18}-material at pH 4.4 (the left part of figures) than at pH 9.4 (the right part). However, this mechanism is not the only one involved, as it does not explain all the data obtained. For instance only a slight pH-dependence of adsorption of pentacaine on both the non-end-capped C_{18}- and end-capped C_{18}-sorbent, a high level of adsorption of all drugs at pH 7.4 when most of the molecules were ionized and thus their adsorption had to be influenced by the absence of silanol groups, etc.

It is evident that end-capping does not eliminate entirely all the residual silanols, and that a small amount of free hydroxyl groups also remains after the reaction with hexamethyldisilazane (HMDS) [19]. However, if these silanols are sufficiently "hidden" to react with relatively small molecules of HMDS, there is only a low probability of them interacting with the larger pentacaine, propranolol and stobadin molecules under the much milder conditions than required by the procedure of end-capping.

These phenomena may be attributable to the existence of not only silanol polar bonds between solute and support. Free electron pairs of siloxane oxygen also can be involved in these types of interactions. In addition, interactions of solutes with water moiety on the solvated silica matrix surface also were suggested [20]. A pictorial representation of the probable interactions is shown in Fig. 4.

As molecules of pentacaine and propranolol differ in the structure of the polar "head", polar interactions can influence their adsorption by different ways, and thus result in their different behaviour towards the C_{18}-support.

Whilst molecules of pentacaine and propranolol can be represented as having a hydrophilic polar "head" and a hydrophobic "tail", the hypothesis of the head-down mechanism of adsorption may be applied, this does not hold for stobadin. The molecules of this drug are more spherical, without a well-distinguished "head" or "tail". Therefore their behaviour on the C_{18}-sorbent has to be different in comparison with that of the previous two drugs. Moreover, dissociation of the aromatic amino group can take place at the lowest pH and thus influence negatively the adsorption of the drug to the end-capped sorbent. However, there is so far no explanation for the optimized adsorption of stobadin at pH 7.4 by end-capping of the sorbent.

Recovery with acetonitrile

It is evident from Figs 1–3 that the elution ability of acetonitrile is inferior to that of methanol, regardless of pH, the nature of the

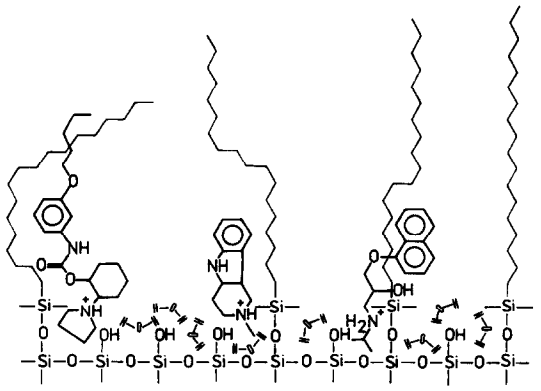


Figure 4
Schematic representation of the adsorption of pentacaine, propranolol and stobadin by C_{18}-silica sorbent.

Table 2
Recovery of pentacaine, propranolol and stobadin expressed as a percentage of the total amount adsorbed to C_{18}-cartridges (A) and end-capped C_{18}-cartridges (B) when washed with acetonitrile

	Recoveries (%)*					
	pH 4.4		pH 7.4		pH 9.4	
Drug	A	B	A	B	A	B
Pentacaine	0	9	1	6	2	5
Propranolol	5	35	0	15	0	4
Stobadin	0	8	0	1	0	0

*Mean, $n = 3$.

drug and the adsorbent. Moreover, in the instance of non-end-capped support, elution of the drugs with 5 ml of acetonitrile was practically nil (Table 2).

Comparing the elution of pentacaine and propranolol, the latter seems to be the more influenced by the absence of silanol groups. End-capping of the support decreased adsorption of propranolol at pH 4.4 and increased its elution with acetonitrile at the same pH more than in the case of pentacaine. Improvement of the elution of all three drugs studied with acetonitrile after end-capping of the support is, however, too low to explain the low elution only by free silanol groups of C_{18}-sorbent. On assuming that there is no great difference in the ability of acetonitrile and methanol to break hydrophobic interactions between solute and sorbent, in addition to interaction with silanol groups, another type of polar interaction must be involved. As mentioned above, siloxane oxygen, having two free electron pairs, and water molecules could serve as the suitable candidates. From the practical point of view, if the analyte also is bound by polar interactions, then acetonitrile may be used as a wash solvent to remove material bound only by hydrophobic interactions.

Conclusions

The results presented in this work, i.e. adsorption of three basic drugs to C_{18}-silica sorbent, their elution with methanol and acetonitrile, corroborated the fact that not all the processes of solute–sorbent interactions between basic drugs and C_{18}-silica can be explained by the partition mechanism itself and that polar interactions also have to be taken into account.

From the point of view of basic drugs, if they can form dissociable "head" and hydrophobic "tail" the "head-to-surface" position is the more preferable. In the instance of more spherical drug molecules, their interaction with the C_{18}-silica support is more complicated, including possibilities from clearly polar (in the case of a dissociated drug and non-C_{18}-covered part of a support) to clearly hydrophobic (in the case of undissociated drug and C_{18}-chain).

Regardless the mechanism of the drug–sorbent interactions, the fact of the low elution ability of acetonitrile towards basic drugs was confirmed. Therefore, the selective solid-phase extraction of basic drugs from C_{18}-sorbent, including the acetonitrile-washing step before elution of drugs, can be utilized for the extraction of basic drugs from biological material. For this extraction, the presence of polar groups in the C_{18}-sorbent is of advantage both for the adsorption of drugs and for their elution (or non-elution) from the support with acetonitrile.

References

[1] L.Šoltés, L. Beneš and D. Berek, *Meth. Find. Exper. Clin. Pharmac.* **5**, 461–465 (1983).
[2] L. Šoltés, D. Berek and M. Štefek, *J. Chromatogr.* **286**, 223–227 (1984).
[3] V. Marko, M. Štefek and L. Šoltés, *J. Chromatogr.* **339**, 410–413 (1985).
[4] V. Marko, J. Wijsbeek and R.A. de Zeeuw, *J. Pharm. Biomed. Anal.* **4**, 333–340 (1986).
[5] V. Marko, *J. Pharm. Biomed. Anal.* **7**, 405–406 (1989).
[6] V. Marko, *Pharmazie* **42**, 387–389 (1987).
[7] V. Marko, *J. Chromatogr.* **433**, 269–275 (1988).
[8] V. Marko, L. Šoltés and T. Trnovec, *Abstr. Int. Symp. Clin. Pharmac.* **1**, 82, Sofia (1986).
[9] P.M. Harrison, A.M. Tonkin, C.M. Cahill and A.J. McLean, *J. Chromatogr.* **343**, 319–338 (1985).
[10] R.J. Ruane and I.D. Wilson, *J. Pharm. Biomed. Anal.* **5**, 723–727 (1987).
[11] Š. Bezek, V. Ščasnár, T. Trnovec, M. Ďurišová, V. Faberová and L. Beneš, *Biopharm. Drug. Dispos.* **7**, 137–150 (1986).
[12] P.H. Wang and E.C. Lien, *J. Pharm. Sci.* **69**, 662–668 (1980).
[13] M. Štefek and L. Beneš, *J. Chromatogr.* **415**, 163–169 (1987).
[14] B. Buszewski, A. Jurášek, J. Garaj, L. Nondek, I. Novák and D. Berek, *J. Liq. Chromatogr.* **10**, 2325–2336 (1987).
[15] K.K. Unger, *Porous Silica.* Elsevier, Amsterdam (1979).
[16] V. Marko, in *Determination of Beta-blockers in Biological Material* (V. Marko, Ed.), pp. 75–96. Elsevier, Amsterdam (1989).
[17] R.D. McDowall, J.C. Pearce and G.S. Murkitt, *J. Pharm. Biomed. Anal.* **4**, 3–21 (1986).
[18] K.K. Unger and K.D. Lork, *Eur. Chromatogr. News* **2**, 14–19 (1988).
[19] L. Nondek and V. Vyskočil, *J. Chromatogr.* **206**, 581–585 (1981).
[20] D.D. Blevins, *Proc. Ann. Int. Symp. Sample Preparation and Isolation Using Bonded Silica, Cherry Hill*, 1986, pp. 299–327.

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