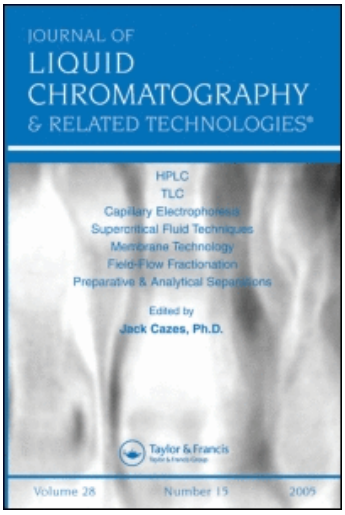

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VARIATIONS IN SOLID-PHASE EXTRACTION OF BASIC DRUGS USING BONDED SILICA. III. PHASE-TO-PHASE VARIATIONS

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ABSTRACT

Phase-to-phase variations of solid-phase extraction of basic drugs were studied using elution profiles obtained after elution of three model drugs from C_{18}-, C_8- and CN-silica. Variations were observed in the extraction of model drugs (in total recovery as well as in the course of elution profiles) and of endogenous compounds from serum. Various types of interactions depending on hydrophobic properties of the drugs and of the silica-modifiers were found to affect the process.. Hydrophobic interactions facilitated not only adsorption but also elution of hydrophobic drugs from hydrophobic sorbents.

INTRODUCTION

Solid-phase extraction (SPE), in principle "digital liquid chromatography" [1], has been used in bioanalysis

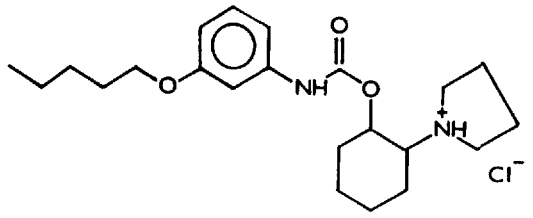
of drugs approximately for ten years. This period has proved sufficient not only for a wide spread of the new sample preparation technology but also for the appearance of the first attempts to summarize the information. In addition to the development of conditions for extraction of individual drugs, efforts to generalize the isolation mode for a broader spectrum of drugs have been observed recently [2,3]. However practically no paper has discussed the choice of phase for efficient SPE. The paper of Musch and Massart [2] favoring the CN-phase in HPLC determination of drugs is a rare exception.

The aim of this paper was to study phase-to-phase variations in SPE of basic drugs. Differences between various phases, i.e. C_{18}-phase, C_8-phase and CN-phase were evaluated from the point of view of polar intractions between solute, sorbent and matrix [4,5] using the same approach as in the first part of this series and including the method of elution profiles. Elution profiles were constructed after elution of model drugs from the sorbents with methanol and acetonitrile. The model basic drugs were pentacaine (pK_a=8.6 [6]), propranolol (pK_a=9.45 [7]) and stobadin (pK_a=8.71 [8]).

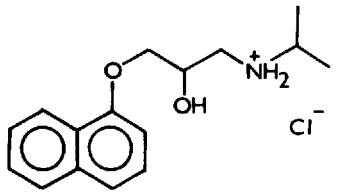
EXPERIMENTAL

Materials

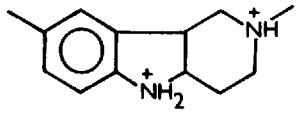
Pentacaine, stobadin and their internal stadards, i.e. the O-hexyl analogue of pentacaine and the N-ethyl analogue of stobadin, were from the same sources as in the preceding papers of the series. Propranolol and its internal standard metoprolol were commercial products (ICI, Macclesfield, Great Britain and Hässle, Mölndal, Sweden).



PENTACAINE



PROPRANOLOL



STOBADIN

FIGURE 1. Chemical structures of pentacaine, propranolol and stobadin

Supelclean LC18, LC8 and LCN SPE tubes, 3 ml volume (the gift of Supelco, Bellefonte, USA) and Bakerbond C_{18}, C_8, and CN SPE columns, 1 ml volume (the gift of Baker Chemikalien, Gross-Gerau, FRG) were used for evaluation of phase-to-phase variation.

All other material used was the same as in the preceding papers of the series.

Extraction Procedure

The extraction columns were conditioned before use by washing them with 2 ml of methanol and 1 ml of water. Afterwards, two types of experiments were carried out to

evaluate adsorption of the drugs to and their elution from the sorbents.

In the first series, 1 µg of the drug studied (pentacaine, propranolol or stobadin) in 1 ml of water was applied to the pre-conditioned columns; the columns were then washed with 1 ml of water. The residual water was displaced from the columns under mild pressure of nitrogen. In the elution step three 1-ml portions followed by one 2-ml portion of methanol or acetonitrile were applied. After the methanolic elution, 1 ml of 5% triethylamine in methanol was used to elute the drugs totally. Individual portions of eluate were collected into 3-ml cone vials containing per 1 µg of a suitable internal standard.

In the second series, 1 µg of the drug was applied to the pre-conditioned columns as 1 ml solution in serum. Washing and elution were then performed as above.

Subsequent processing of the eluates, i.e. evaporation, reconstitution in ethyl acetate and, if necessary, derivatization, were performed as described in the first paper of the series.

For the evaluation of the selectivity of the C_{18}, C_8 and CN sorbents towards endogenous compounds from serum, 1 ml of human blank serum was applied to the conditioned columns. The columns were washed with 1 ml of water, residual water was displaced with mild pressure of nitrogen and andogens retained in the columns were eluted with 3 ml of methanol. Methanol was evaporated, 250 µl of ethyl acetate was added to the residue and 3 µl of the solution was analyzed gas chromatographically under temperature programmed conditions.

Instrumentation

Capillary gas-liquid chromatography was used for the determination of the recovery of pentacaine and sto-

badin eluted from the columns under the same conditions as in the preceding papers. For the determination of propranolol the following conditions were used: the temperature of the direct injection port and of the thermionic nitrogen detector was 300°C, that of the column 185°C. The other conditions remained unchanged.

Gas chromatographic separation of the samples after SPE of blank serum was achieved under temperature programmed conditions using the following sequence: initial temperature 100°C for 0.01 min; program at 10°C/min to 250°C and hold for 5 min.

RESULTS AND DISCUSSION

In comparison with conventional liquid-liquid extraction, solid-phase extraction provides a much more complex system where several factors may contribute to the results observed. In the case of modified silica sorbents, there are some indications of different adsorption mechanisms of non-polar and polar basic molecules. While the non-polar molecules appear to interact most favorably near the centre of solvated chains, basic molecules penetrate deeply to interact with acidic sites of the silica surface [4]. Since acidic sites are present in all types of modified silica, in addition to hydrophobic interactions a presence of polar interactions between solute and sorbent is expected in solid-phase extraction of basic drugs regardless the type of the sorbent.

Figure 2 shows a comparison of elution profiles obtained after the methanol elution of pentacaine, propranolol and stobadin from Supelclean cartridges packed with different sorbent, i.e. with the C_{18}-, C_8- and CN-modified silica. The matrix which the drugs were ex-

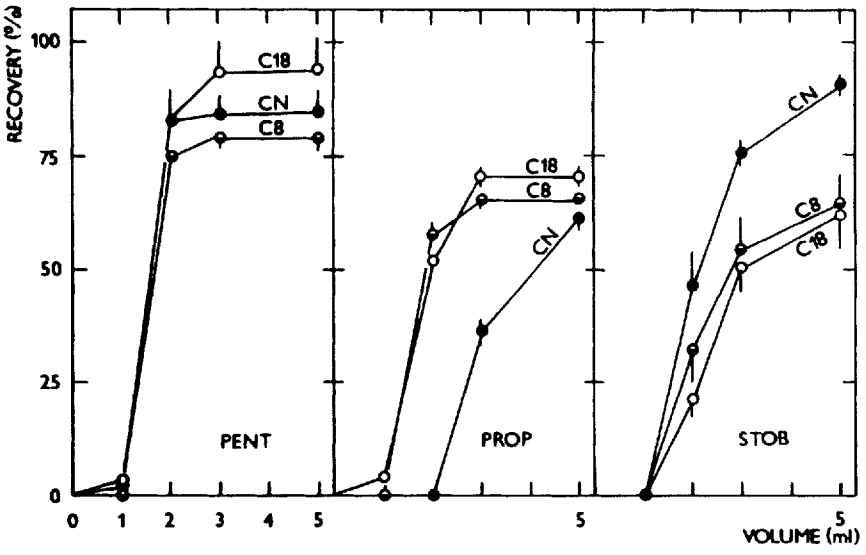


FIGURE 2. Elution profiles of pentacaine (PENT), propranolol (PROP) and stobadin (STOB); solid-phase extraction from serum using Supelclean LC18, LC8 and LCN cartridges; elution with methanol.

tracted from was human serum. There are differences in the extraction of the same drug by different sorbents as well as in the extraction of different drugs by the same sorbent. The differences are both in the courses of the extraction profiles and in the total recovery (Table 1). For the hydrophobic drugs pentacaine (log P = 4.69 [6]) and propranolol (log P = 3.17 [9]), the most hydrophobic sorbent of the three tested, i.e. the C_{18}-silica, proved to have better properties in comparison with the other two sorbents, while the less hydrophobic stobadin (log P = 1.27 [10]) was best adsorbed to and eluted from the cyanopropyl-modified silica. The best suitability of the C_{18}-silica for solid-phase extraction of the hydro-

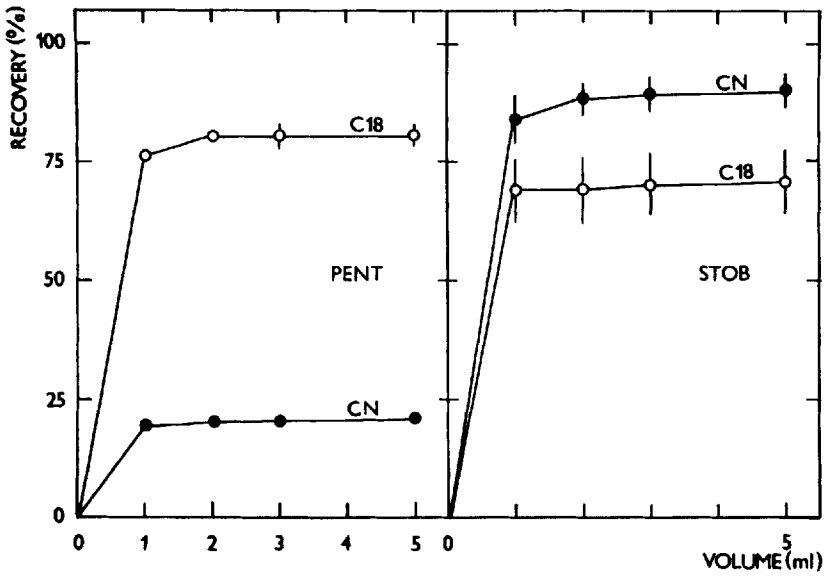


FIGURE 3. Elution profiles of pentacaine (PENT) and stobadin (STOB); solid-phase extraction from serum using Bakerbond C18 and CN cartridges; elution with methanol.

phobic pentacaine and of the CN-silica for SPE of stobadin was demonstrated also in the methanol elution profiles of the drugs using 1 ml Bakerbond cartridges (Figure 3).

Despite the small size of the group of drugs tested the results obtained from the methanol elution profiles prove the existence of interactions between the drugs and the sorbent dependent on hydrophobic properties of the drugs, i.e. non-polar interactions. On the other hand, practically nil elution of the drugs with acetonitrile (Table 1) indicates the presence of polar solute-sorbent interactions in the case of each type of modified silica.

TABLE 1

Recoveries of Solid-Phase Extraction of Pentacaine, Propranolol and Stobadin with Methanol (MeOH) and Acetonitrile (MeCN) from Serum Using Cartridges with Different Sorbents

Sorbent	Recoveries [%]					
	Pentacaine		Propranolol		Stobadin	
	MeOH	MeCN	MeOH	MeCN	MeOH	MeCN
C_{18}	94.3±3.5	1.1±0.5	70.8±0.9	0	69.3±7.0	0
C_8	79.3±2.9	1.2±1.0	65.9±1.2	0	64.6±9.8	0
CN	84.7±4.7	2.8±1.9	61.2±1.1	0	90.7±2.2	0

In addition to solute-sorbent interactions, other interactions, e.g. matrix-solute and matrix-sorbent, are also involved in the elution profiles in Figures 2 and 3. In the effort to simplify the extraction by removing these interactions, we compared the methanol elution profiles obtained after extraction of the three drugs from distilled water (Figure 4).

Two types of elution behavior are visible from the Figure. The first type is demonstrated on the elution profiles of pentacaine and propranolol, the second type is observed on the elution profiles of stobadin. The hydrophobic drugs pentacaine and propranolol were expressively better eluted from the bonded silica with hydrophobic chains (C_{18}, C_8) than from that with cyanopropyl groups. Moreover, interactions between the drugs and the CN-sorbent were so strong that not even the elution with 1 ml of 5% triethylamine in methanol following that with 5 ml of pure methanol achieved removal of the whole amount of the drugs from the sorbent (Table 2).

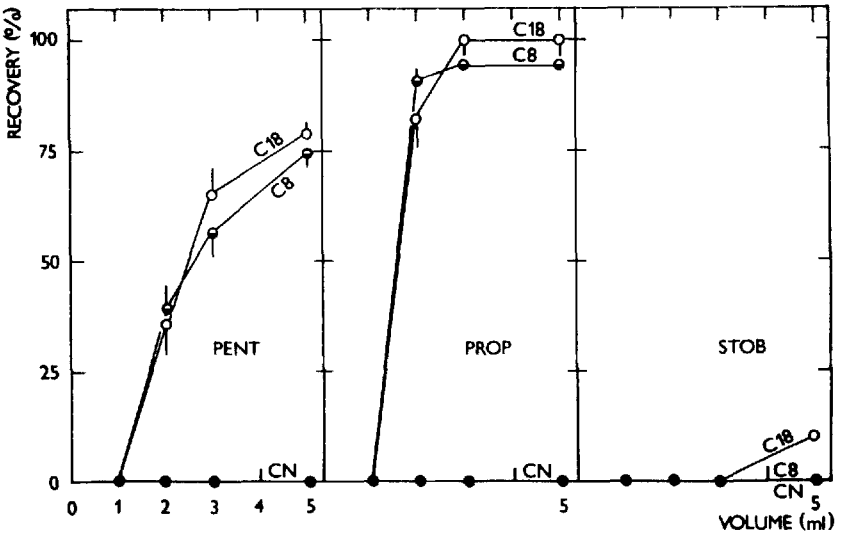


FIGURE 4. Elution profiles of pentacaine (PENT), propranolol (PROP) and stobadin (STOB); solid-phase extraction from water using Supelclean LC18, LC8 and LCN cartridges; elution with methanol.

TABLE 2

Recoveries of Solid-Phase Extraction of Pentacaine, Propranolol and Stobadin from Water Using Cartridges with Different Sorbent

Sorbent	Recoveries [%]		
	Pentacaine	Propranolol	Stobadin
C_{18}	87.2±2.9	99.8±3.7	56.1±0.8
C_8	80.0±3.1	94.2±1.0	69.3±0.5
CN	58.3±2.0	49.8±0.2	70.0±1.0

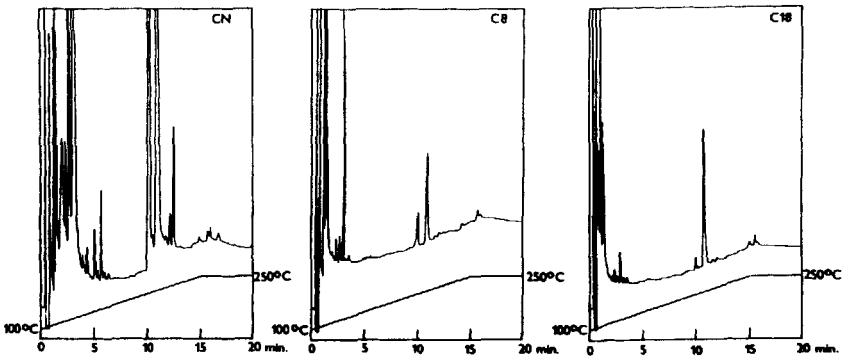


FIGURE 5. Gas chromatograms of human serum blanks after their solid-phase extraction with different types of Supelclean LC cartridges.

Comparison of the elution profiles obtained with CN-modified silica after elution of pentacaine and propranolol from water (Figure 4) and from serum (Figure 2) clearly shows the influence of endogens from serum on solute-sorbent interactions. This influence was discussed in detail in the first part of the series.

In the case of the hydrophilic stobadin, its elution with 5 ml of methanol was practically nil from all the three types of bonded silica and therefore no substantial phase-to-phase variations could be demonstrated in the range studied. This indicates the presence of one source of strong interactions for the modified silicas regardless the substituent type of silanol groups, i.e. interactions caused by polar groups situated on the silica surface. The influence of endogens from serum on the considerable improvement of the elutions of stobadin from this matrix in comparison with elutions from water (Figures 2 and 4) corroborate the above given findings.

The results indicate that hydrophobic interactions between "tails" of hydrophobic pentacaine and propranolol and octadecyl or octyl chains of C_{18}- or C_8-silica can facilitate elution of the drugs from the sorbents. The lack of sources for these interactions, due either to properties of the sorbent (short cyanopropyl chain) or of the drug ("non-tailed" hydrophilic stobadin) decreases elution.

Another problem that arises in connection with phase-to-phase variations in solid-phase extraction is the ability of different phases to extract or not to extract endogenous compounds from biological matrices and thus to influence a signal-to-noise ratio of subsequent analytical methods. Figure 5 shows chromatograms of human serum blanks after their extraction with different types of Supelclean LC cartridges and capillary GLC analysis. As seen in the Figure, there is a difference between the chromatograms of the extracts from the C_{18}- and C_8-sorbent on the one hand and the extract obtained after extraction using the CN-sorbent on the other hand. Considerably more peaks are observable in the chromatogram after CN-extraction.

To summarize the results, in addition to polar interactions, various types of interactions depending on hydrophobic properties of drugs as well as on silica--modifiers were demonstrated in SPE of basic drugs. Hydrophobic interactions between drugs containing a hydrophobic "tail" (pentacaine, propranolol) and hydrophobic sorbents (C_{18}, C_8) facilitated not only adsorption to, but also elution from the C_{18} and C_8-sorbents. A lack of these interactions can cause difficulties in the elution step. The differences in the behavior of hydrophobic and hydrophilic drugs are better manifested in their solid--phase extraction from water than from serum, since in

the latter serum endogens mask polar goups of the silica surface and thus facilitate elution of drugs.

In addition to phase-to-phase variations in SPE of drugs, also variations of other compounds involved, e.g. endogens from serum, were shown to influence the quality of the extraction.

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