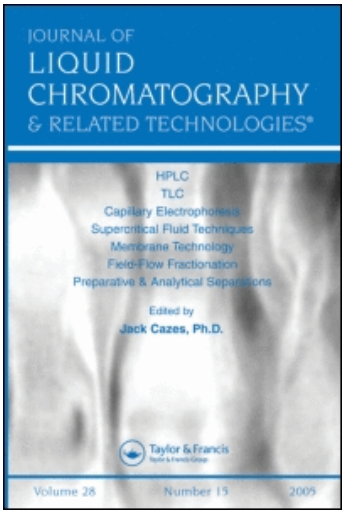

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VARIATIONS IN SOLID-PHASE EXTRACTION OF BASIC DRUGS USING BONDED SILICA. II. BATCH-TO-BATCH VARIATIONS

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ABSTRACT

Batch-to-batch variations of solid-phase extraction of basic drugs from serum and water were studied using different batches of C18 sorbent by means of methanol and acetonitrile elution profiles of two model drugs. Variations were observed mainly in adsorption of the drugs. Differences between the batches in dependence on the matrix which the drugs were extracted from (water, serum) indicated different accessibility of polar groups of sorbents to form interaction with the analytes.

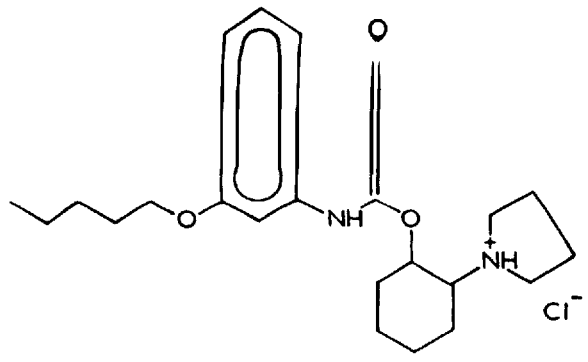
INTRODUCTION

The introduction of solid-phase extraction (SPE) represented a major innovation in transforming biologi-

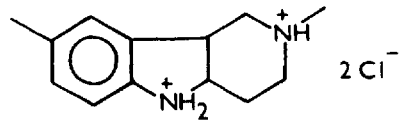
cal samples into analytical ones. Many solid-phase adsorbents are currently available, generally based on silica gel and its various alkyl modifications, to give a variety of bonded phases. Despite several advantages that SPE offers to bioanalysts in comparison with liquid-liquid extraction (convenience, flexibility and relative ease of automation [1]), SPE methodology has been slow to gain acceptance, due in part to the perceived expense of the cartidges and in part to a lack of reproducibility. Although one source of the irreproducibility is inadequate information and experience in the technique among users, variations in material produced for SPE definitely play an undeniable role.

During an approximately ten-year experience acquired in the authors' laboratories with solid-phase extraction of drugs, reproducibility of material for SPE has been the major complaint discussed and problem to be solved. Two sources of this irreproducibility have been considered as the main ones, i.e. manufacturer-to-manufacturer variations, discussed in the first part of this series, and batch-to-batch or lot-to-lot variations, to be dealt with in this part.

In the study of batch-to-batch variations, the same procedure as in the previous part was used, i.e. evaluation of elution profiles obtained after SPE of model basic drugs - pentacaine (pK_a=8.6 [2]) and stobadin (pK_a=8.71 [3]) (Fig. 1) - from water and serum and their elution with methanol and acetonitrile. Our previous findings on polar interactions between solute and sorbent [4,5] served as theoretical background and water--to-serum and methanol-to-acetonitrile differences in elution profiles as starting points for the evaluation.



PENTACAINE



STOBADIN

FIGURE 1. Chemical structures of pentacaine and stobadin

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 donators as in the preceding paper of the series.

Separcol SI C18 extraction minicolumns (lots 3738, 384, 551, and 552) were from the Polymer Institute, Slovak Academy of Sciences, Bratislava, CSFR.

Analytical grade methanol and ethyl acetate were obtained from Lachema, Brno, CSFR, acetonitrile (puriss. p.a.) and trimethylanilinium hydroxide (p.a., 0.1 mol/l in methanol) were purchased from Fluka, Buchs, Switzerland. Triethylamine (Laborchemie, Apolda, GDR) was distilled before use.

All glassware was cleansed in hydrochloric acid, then silanized with a 5% solution of Surfasil (Pierce, Oud Beijerland, The Netherlands) in benzene.

Human serum was from the Department of Hematology and Transfusiology, School of Medicine, Comenius University, Bratislava, CSFR.

Extraction procedure

The extraction columns 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 (1 µg of pentacaine or stobadin in water or serum). After passage of the sample 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 aplied. After methanolic elution 1 ml of 5% triethylamine in methanol were used to elute the drugs totally [5]. 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.

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.

Instrumentation

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

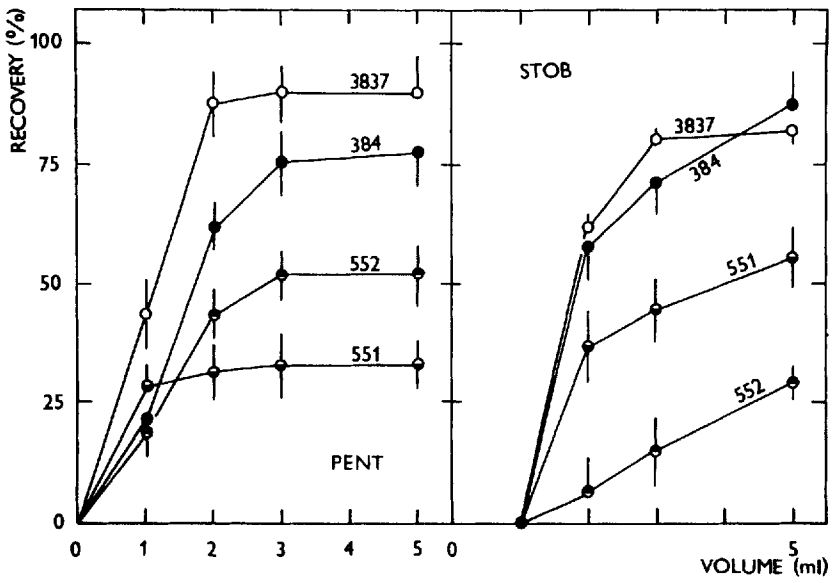


FIGURE 2. Elution profiles of pentacaine (PENT) and stobadin (STOB); solid-phase extraction from serum using different batches of Separcol SI C18 cartridges; elution with methanol.

badin eluted from the columns under the same conditions as in the first part of the series.

RESULTS AND DISCUSSION

Four batches of Separcol SI C18 were compared on the basis of methanol elution profiles obtained in extraction of pentacaine and stobadin from serum (Figure 2). In both panels of the figure, great differences can be observed indicating significant batch-to-batch variations in different lots of Separcol SI C18 cartridges.

TABLE 1

Recoveries of Solid-Phase Extraction of Pentacaine and Stobadin from Serum Using Different Batches of Separcol SI C18 Cartridges

Batch	Recovery [%]	
	Pentacaine	Stobadin
3738	90.1± 7.2	86.7± 1.5
384	78.3± 8.5	98.2± 6.9
551	35.9± 1.0	57.1±12.1
552	53.4±10.1	34.6± 3.9

A closer view on the elution profiles reveals that the differences are caused by different adsorption of the drugs while elution of the drugs adsorbed was very similar. Recovery of the extractions was in a wide range for both drugs, i.e. from 36% to 90% for pentacaine and from 35% to 98% for stobadin (Table 1).

On the basis of their adsorption and elution properties, the bathes can be divided into two groups. The first group is formed by batches 3738 and 384, i.e. the batches whose properties enabled them to serve as sorbents for SPE of basic drugs from serum. Properties of the members of the second group (batches 551 and 552), mainly their low adsorption ability, exclude them from utilization in SPE of basic drugs from serum.

Elution profiles obtained after elution of pentacaine and stobadin with acetonitrile (Figure 3) reveal a separate position of the lot 551 among the others. This lot was the only one which allowed to elute the adsorbed drugs also by acetonitrile, i.e. by an eluent

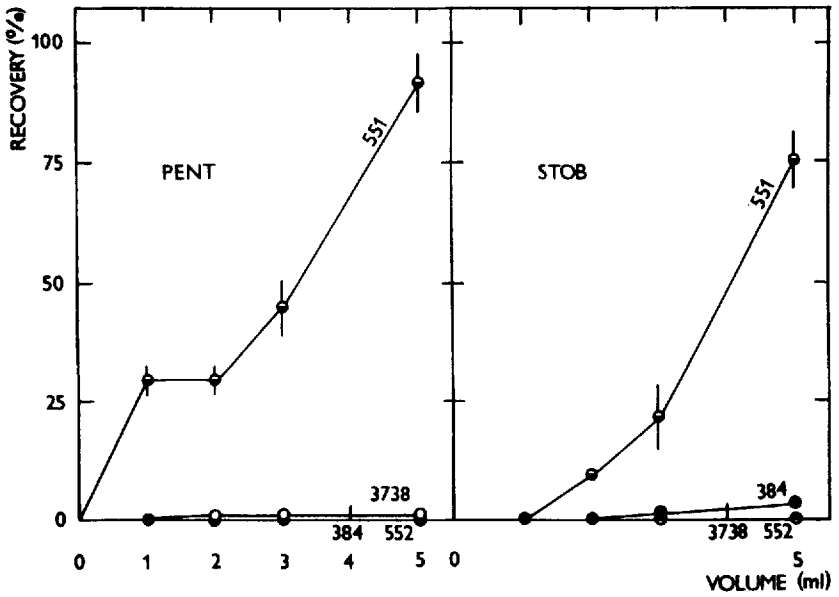


FIGURE 3. Elution profiles of pentacaine (PENT) and stobadin (STOB) adsorbed (relative elution profiles); solid-phase extraction from serum using different batches of Separcol SI C18 C_{18}-cartridges; elution with acetonitrile.

with low proton-aceptor properties [5]. This fact indicates the lack of sources for polar interactions, i.e. the lack of polar groups of the sorbent, accessible for basic drugs.

Quite different and somewhat surprising results were obtained in the experiment in which not serum but water was the matrix the drugs were extracted from (Figure 4). For this experiment two batches, one from each group (lots 384 and 551), were taken. The differences are observable in adsorption, as well as in elution properties of the sorbents (the recoveries are listed in Table 2).

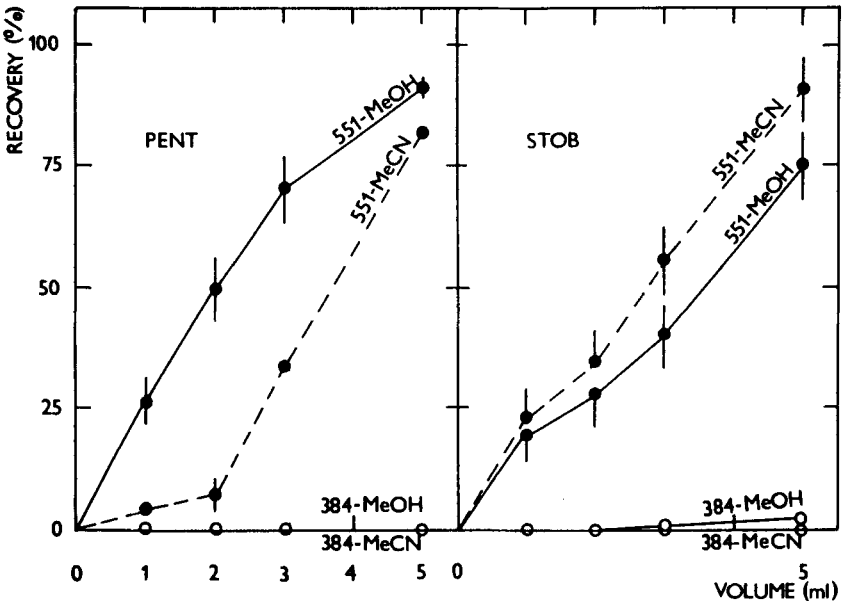


FIGURE 4. Elution profiles of pentacaine (PENT) and stobadin (STOB); solid-phase extraction from water using different batches of Separcol SI C_{18}-cartridges; elution with methanol (MeOH) and acetonitrile (MeCN).

TABLE 2

Recoveries of Solid-Phase Extraction of Pentacaine and Stobadin from Water Using Different Batches of Separcol SI C18 Cartridges

Batch	Recovery [%]	
	Pentacaine	Stobadin
384	80.8±2.0	72.0±0.1
551	97.5±1.3	99.9±9.8

Unlike the extraction from serum, it was lot 551 that adsorbed a significantly higher amount of the drugs from water than did lot 384. Moreover, the drugs adsorbed on the sorbent of the batch 384 were not eluted either with 5 ml of acetonitrile or methanol (Figure 4). Only solution of triethylamine in methanol was able to break strong solute-sorbent interactions in this batch. On the other hand, the drugs retained by the C_{18}--sorbent of batch 551 were well eluted not only by methanol but also by acetonitrile. This fact, together with the low adsorption ability of the batch on extracting the drugs from serum indicates that the main type of interactions between the basic drugs tested and the C_{18}-sorbent 551 are hydrophobic interactions. These low--energy interactions are sufficient for extraction of the drugs from water. On the contrary, when serum is the matrix the drugs are extracted from, these properties are insufficient for breaking solute-matrix interactions. Moreover, a part of hydrophobic groups is masked by endogens from serum. These facts account for the low adsorption of basic drugs from serum.

The lack of polar groups of the batch 551 on the one hand and a high level of hydrophobic interactions (compare adsorption of pentacaine and stobadin from water on the batches 384 and 551 in Table 2) of this batch on the other hand could be signs of a higher covering of the silica surface by C_{18}-groups in the batch 551 in comparison with the others. This hypothesis, however, failed to be confirmed by the content of carbon as a marker of the silica covering by C_{18}-groups (Table 3). The C_{18}-sorbent of the batch 551 contained the lowest amount of C of all the batches tested.

For an explanation of the unusual behavior of the C_{18}-silica sorbent of the batch 551, a hypothesis of various conformations of alkyl-chains in C_{18}-sorbents

TABLE 3

Content of Carbon and Specific Area of Separcol SI C18 Cartridges of Different Batches.

Batch	Content of Carbon [%]	Specific Area [m^2/g]
3738	22.09	176
384	21.82	163
551	20.81	178
552	20.99	177

can be taken into account. According to this hypothesis, two conformations of saturated hydrocarbon chains bonded to silica can occur. One conformation would be associated with stiffened chains of little mobility (brush model), the other one with bent chains of higher mobility (droplet model) [6]. If the hypothesis is applied to the sorbents tested, then those with "normal" behavior, i.e. the batches 3738, 384, and probably also 552, contain C_{18}-chains mainly in the "brush" conformation with polar groups of the silica accessible for polar interactions with basic drugs. On the contrary, in the remaining sorbent, i.e. the batch 551, the majority of the alkyl chains is in the "droplet" conformation, making the surface hydrophobic and covering polar groups of the silica capable to form higher energy interactions. This batch-to-batch difference could be caused by an unintended change in the procedure of covering the silica by alkyl chains.

When judging the observed batch-to-batch variations from a practical point of view, then water-to-serum dif-

ferences indicate unambigously the need for different C_{18}-sorbents for SPE from different matrices.

In conclusion, the first source of irreproducibility in SPE, i.e. manufacturer-to-manufacturer variations, can be relatively easily eliminated by using extraction columns or cartridges of one vendor. On the contrary, for a user it is practically impossible to avoid batch-to-batch variations. The only way is to test each batch of the sorbent obtained. Every laboratory can create its own control system, however we recommend the formation of methanol and acetonitrile elution profiles, since on their basis one is capable to evaluate the quality of a multimodal complex of interactions.

Full improvement can be achieved only by producers of material for SPE, and that by unification of starting silicas and of procedures for preparation of their modified types. Moreover, further study of solute-sorbent interactions in SPE can reveal principles of these interactions leading to preparation of different types of sorbents dedicated for SPE of different drugs from different matrices.

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