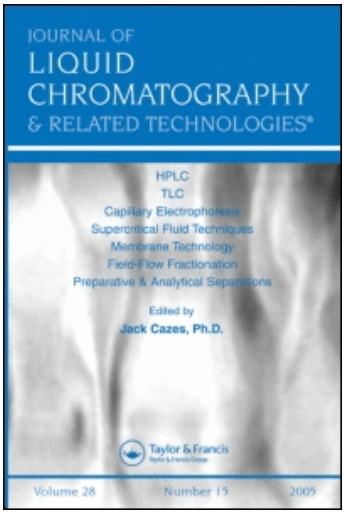

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VARIATIONS IN SOLID-PHASE EXTRACTION OF BASIC DRUGS USING BONDED SILICA. I. MANUFACTURER-TO-MANUFACTURER VARIATIONS

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ABSTRACT

Manufacturer-to-manufacturer variations of solid-phase extraction (SPE) of basic drugs from serum and water using C_{18}-silica were studied and solute-sorbent interactions discussed on the basis of elution profiles obtained after elution of two model drugs from C_{18}-sorbents of five vendors. In addition to manfacturer-to-manufacturer variations, differences were observed also after SPE from water and from serum indicating an inclusion of endogens from serum to processes run in SPE. Differences in polar properties of the sorbents were found to be the main cause of the variations.

INTRODUCTION

Over the last decade sample preparation has been gaining ground as one of the most important fields in bioanalysis of drugs. At the beginning of the eighties,

the application of knowledge from bonded silica chemistry transferred from HPLC column technology to sample preparation was the main step in breaking through the fossilized state in changing a biological sample to an analytical one. This resulted in the appearance of an alternative approach to solvent extraction, called solid-phase extraction (SPE) or liquid-solid extraction, based on principles of reversed-phase liquid chromatography. The recent trend shows the use of chemically modified silicas for sample preparation. The material for SPE is available from an increasing number of manufacturers, which, together with an increasing number of papers dealing with the utilization of modified silicas for sample preparation, indicates a real trend rather than a fad. So far some tens of different drugs have been isolated by this type of extraction [1,2].

As advantages of SPE in comparison with liquid-liquid extraction, easy handling, saving in the extraction time, and the possibility for on-line arrangement and automation are those referred to most frequently. On the other hand, there is one disadvantage of SPE which can sometimes counterbalance all the advantages mentioned above and which is possibly the main brake on an even faster development of SPE, namely the variability of modified silica used as material in SPE. In addition to manufacturer-to-manufacturer variations referred to frequently [3,4], batch-to-batch variations have been observed as well [1,5].

The aim of this paper was to study the former variations, i.e. manufacturer-to-manufacturer variations observable in SPE of basic drugs. Knowledge on polar interactions involved in SPE, obtained in our previous studies [6,7] forms a theoretical background of this work. The quality of extraction procedure was determined, different cartridges were compared and possible

solute-sorbent interactions discussed on the basis of elution profiles obtained after elution of model drugs from the sorbents with methanol and acetonitrile. The methanol-to-acetonitrile difference in elution ability towards basic drugs renders a convenient starting point to discussion on different types of solute-sorbent interactions [6,7]. The model basic drugs used in the study were those which had proved convenience [6,7], i.e. pentacaine (pK_a=8.6 [8]) and stobadin (pK_a=8.71 [9]) (Fig. 1).

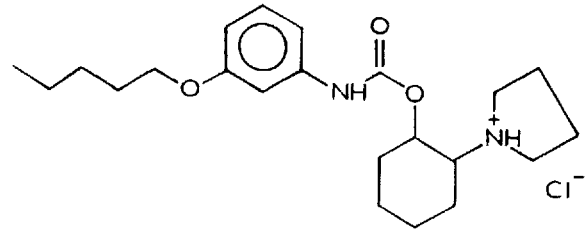
EXPERIMENTAL

Materials

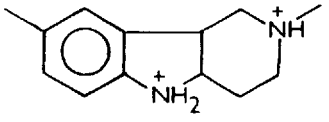
Pentacaine was kindly supplied by the manufacturer, Galena, Opava, CSFR, its internal standard, the O-hexyl analogue, was synthesized at the Faculty of Pharmacy, Comenius University, Bratislava, CSFR. Stobadin and its internal standard, the N-ethyl analogue, were prepared at the Institute of Organic Chemistry and Biochemistry, Prague, CSFR.

Cartridges for SPE were gifts of the following vendors: Baker Chemikalien, Gross-Gerau, FRG (Bakerbond C_{18} SPE columns, 1 ml volume), Macherey-Nagel, Düren, FRG (Chromabond C18 extraction columns, 3 ml volume), Polymer Institute, Slovak Academy of Sciences, Bratislava, CSFR (Separcol SI C18 minicolumns, 3 ml), Supelco, Bellefonte, USA (Supelclean LC18 tubes, 3 ml volume and 1 ml volume), Waters, Milford, USA (Sep-Pak C18 cartridges, 3 ml volume).

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



PENTACAINE



STOBADIN

FIGURE 1. Chemical structures of pentacaine and stobadin

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

Cartridges were conditioned by flushing with 2 ml methanol and 1 ml water. Then 1 ml of serum or water spiked with 1 µg pentacaine or stobadin was applied. After passage of the sample through the cartridge, the sorbent was washed with 1 ml water. The residual water

was displaced from the cartridge under mild pressure. For the elution of the retained drug, 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 [7]. Individual portions of the eluate were collected into 3-ml cone vials containing per 1 µg of a suitable internal standard. The solvent was evaporated to dryness at 55°C under nitrogen, 250 µl of ethyl acetate were added to the dry residue and the vials were agitated on a Vortex for 10 sec. The solution was placed into autosampler vials and 3 µl of the solution were injected into the gas chromatograph. Pentacaine and its internal standard were methylated before analysis [10], stobadin was analyzed directly [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. The evaluations were carried out in triplicate.

Instrumentation

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 Model 7673A Hewlett-Packard Autosampler. The chromatograph was equipped with a thermionic nitrogen detector (NPD) and a direct injection port. 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 injector port and of the detector was 300°C, that of the column 220°C and 145°C for the deter-

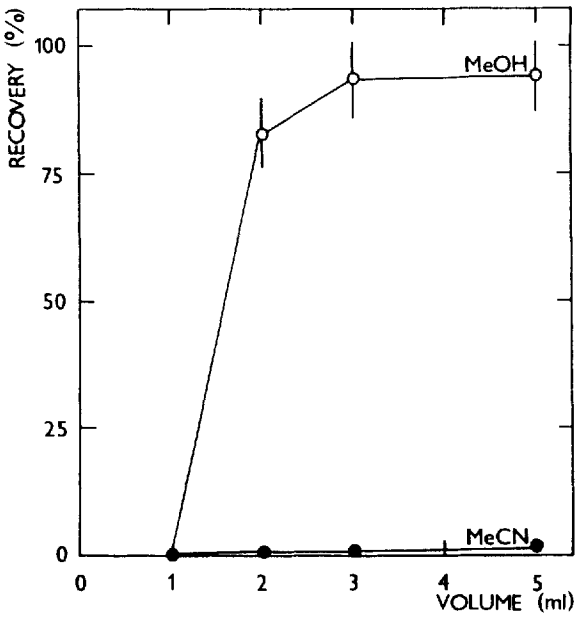


FIGURE 2. Elution profiles of pentacaine; solid-phase extraction from serum using Supelclean LC18 cartridges; elution with methanol (MeOH) and acetonitrile (MeCN).

mination of pentacaine and stobadin, respectively. Nitrogen used as a carrier gas was maintained at 25 ml/min; no auxilliary gas was used. Purge activation time was 30 sec.

RESULTS AND DISCUSSION

An example of elution profiles obtained after elution of a basic drug from a modified silica with methanol and acetonitrile is given in Fig. 2. These elution profiles were obtained after elution of pentacaine from Supelclean C18 cartridges in extraction of the drug from

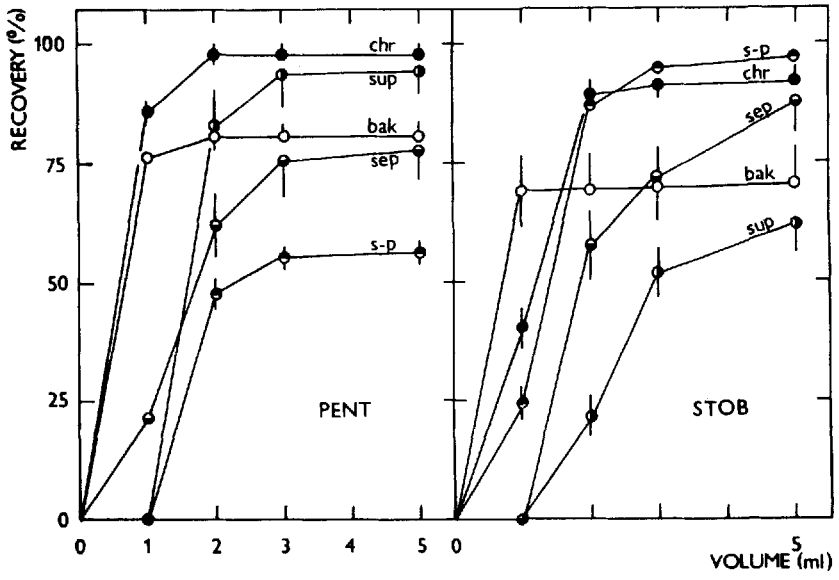


FIGURE 3. Elution profiles of pentacaine (PENT) and stobadin (STOB); solid-phase extraction from serum using different C_{18}-cartridges (bak - Bakerbond, chr - Chromabond, sep - Separcol, s-p - Sep-Pak, sup - Supeclean); elution with methanol.

human serum. The different courses of the two curves demonstrate the different ability of the eluents to break polar interactions between solute and sorbent, given by differences in proton-acceptor properties of the eluents [7]. From a practical point of view, this difference has been utilized for selective solid-phase extraction (SSPE) of basic drugs in our laboratory for more than five years [6].

Figure 3 shows comparison of elution profiles obtained after elution of pentacaine and stobadin from C18-cartridges of different manufacturers with methanol. The matrix which the drugs were extracted from was, as

TABLE 1

Recoveries of Solid-Phase Extraction of Pentacaine and Stobadin from Serum Using Different C_{18}-Cartridges

Cartridge	Recovery [%]	
	Pentacaine	Stobadin
3 ml cartridges		
Separcol	78.3±7.5	98.2±6.9
Chromabond	97.8±5.2	92.1±2.7
Supelclean	94.3±3.5	69.3±7.0
Sep-Pak	56.3±2.0	97.8±0.3
1 ml cartridges		
Baker	80.6±2.8	70.5±6.2
Supelclean	60.9±0.2	100.0±1.8

previously, human serum. In the figure great differences can be observed in the course of extraction profiles, i.e. in the kinetics of elution of the drugs from the sorbent. However, the differences are manifested not only in the course of the curves, but also in the final levels reached, i.e. in total recoveries of the elutions. Using 3 ml cartridges the recoveries ranged in the pentacaine extractions from 56% (Sep-Pak) to 97% (Chromabond) and in the stobadin extractions from 69% (Supelclean) to 98% (Separcol) (Table 1). Comparing the two 1 ml cartridges, i.e. Bakerbond and Supelclean, the difference was manifest as well.

Differences were observed also on comparing cartridges of different manufacturers and different amounts

of sorbent (Supelclean 3 ml, Supelclean 1 ml and Baker-bond 1 ml cartridges in Table 1). These results can serve as an example demonstrating that not the amount of sorbent but its quality plays the major role in SPE of basic drugs.

The main conclusion which can be drawn from the point of view of practical utilization of the results presented is that there is no good or bad sorbent. The sorbent which manifests good properties towards one basic drug can be unfit in extraction of another one. The recoveries of the Supelclean LC18 3-ml cartridges in pentacaine and stobadin extractions (Table 1) can serve as an example for this statement.

The obtained findigs made us adress the question: What is responsible for the differences observed - are they differences in covering silica surface with alkyl chains or differences of the silica surface itself. Except the Sep-Pak cartridges, all SPE columns have a very similar carbon content, i.e. similar covering of the surface, and all of them are endcapped [4]. Therefore, the answer to the question on the origin of manufacturer-to-manufacturer difference is to be sought elsewhere.

The elution profiles in Fig. 4 can provide an answer. This figure shows elution profiles obtained under the same conditions as the elution profiles in Fig. 3. The only difference is that the elution profiles in Fig. 4 were obtained in extraction of pentacaine from water and not from human serum. In the case of all the cartridges used, significantly shallower elution profiles were obtained compared to SPE from serum, while the total recoveries were not substantially affected (Table 2). One of the possible explanations for the water-to--serum differences is that endogenous compounds from serum occupy polar sites of the silica surface and thus reduce the possibility to form polar interactions be-

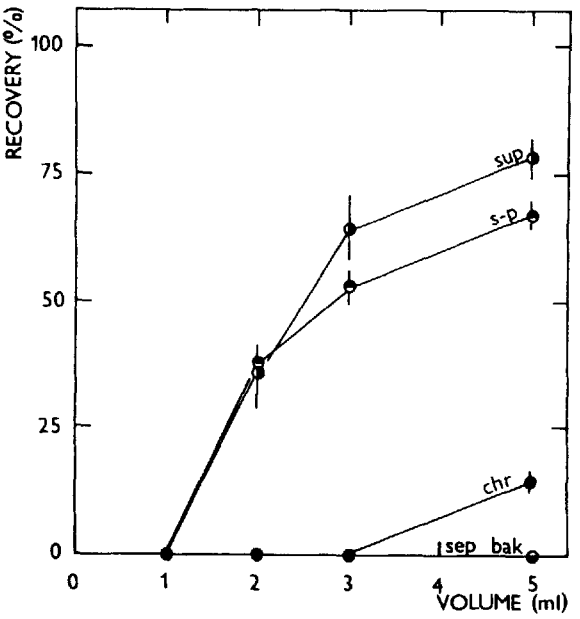


FIGURE 4. Elution profiles of pentacaine; solid-phase extraction from water using different C_{18}-cartridges (bak - Bakerbond, chr - Chromabond, sep - Separcol, s-p - Sep-Pak, sup - Supelclean); elution with methanol.

TABLE 2

Recoveries of Solid-Phase Extraction of Pentacaine from Water Using Different C_{18}-Cartridges

Cartridge	Recovery [%]
Baker 1 ml	93.4±1.1
Separcol 3 ml	80.8±4.3
Chromabond 3 ml	98.3±2.5
Supelclean 3 ml	87.2±2.9
Sep-Pak 3 ml	67.4±3.8

tween a sorbent and a drug. Therefore application of pentacaine in water makes the elution of the drug more difficult than its application in serum. These findings, reported also for other drugs [11], turn the attention to polar interactions between solute and sorbent and thus to properties of the silica itself.

The problem of polar interactions and the effect of endogenous compounds from serum on these processes was studied more extensively using Supelclean LC18 cartridges. Two types of experiments were carried out.

In the first series, 1 μg of the drug studied (pentacaine or stobadin) in 1 ml of water was applied onto cartridges after their conditioning, then the cartridges were washed with 1 ml of water, and methanol or acetonitrile was used to form elution profiles.

In the second series, 1 ml of human serum was passed through the cartridges after conditioning, the cartridges were washed with 1 ml of water, and the solution of the drug in water was applied afterwards.

The methanolic elution profiles are shown in Figure 5. In both cases, i.e. in the elutions of pentacaine as well as stobadin from the C_{18}-silica, the application of blank serum before the application of the water solutions of the drugs facilitated their elution by methanol. It was manifested by an "erection" of the elution profiles. Moreover, in the first series polar interactions between the sorbent and the drugs were so strong that it was impossible to break them all and thus to elute the drug totally even by using 6 ml of methanol with the addition of 5% of triethylamine to the last 1-ml portion (87% and 56% recovery of pentacaine and stobadin, respectively, Table 3). This however was not the case in the second series after "pre-conditioning" of the sorbents with serum (91% and 92% for pentacaine and stobadin, Table 3).

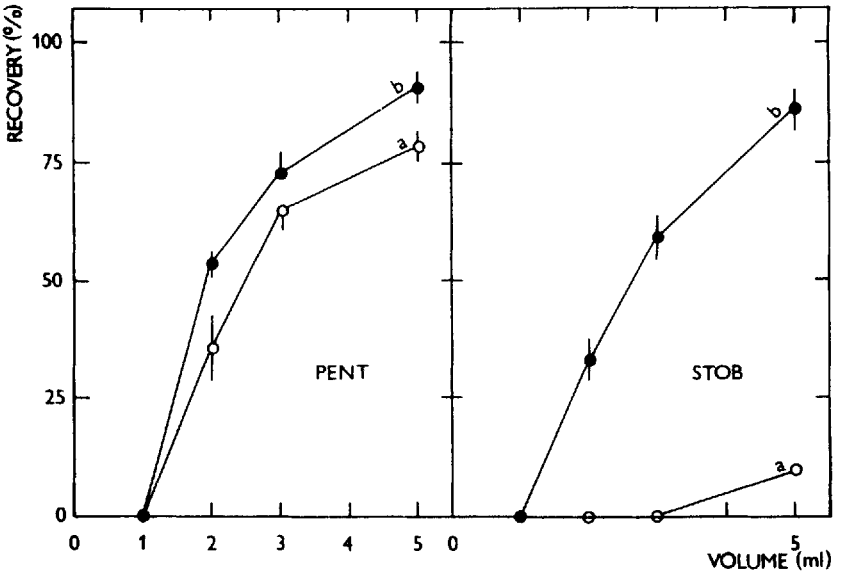


FIGURE 5. Elution profiles of pentacaine (PENT) and stobadin (STOB); solid-phase extraction from water (a) and from water after application of blank serum (b); Supelclean LC18 cartridges; elution with methanol.

TABLE 3

Recoveries of Solid-Phase Extraction of Pentacaine and Stobadin from Water without and with "Pre-Conditioning" of the C_{18}-Silica Sorbent with Serum after Elution with Methanol (MeOH) and Acetonitrile (MeCN)

Drug	Recovery [%]			
	Without Serum		With Serum	
	MeOH	MeCN	MeOH	MeCN
Pentacaine	87.2±2.9	0.0	91.4±2.6	0.0
Stobadin	56.1±0.8	0.0	91.8±4.5	0.0

The whole situation is complicated when taking into account elution profiles of the drugs after elution with acetonitrile. The assumption that masking polar sites of the sorbent by endogens from serum facilitates elution as such, regardless the eluent used, proved to apply only partly, i.e. in the elution with methanol. The elution with the second eluent used, acetonitrile, was not affected at all by "pre-conditioning" with serum, and recoveries of the drugs were nil in both series (Table 3).

The fact that the application of serum before the application of basic drugs to C_{18}-sorbent in water solution facilitates the elution of the drugs only with methanol and does not influence that with acetonitrile causes that the hypothesis on a simple masking of the polar sites by endogens from serum is not fully sufficient for explaining the whole process.

In conclusion, the main cause of manufacturer-to--manufacturer variations in SPE of basic drugs by C_{18}--silica are differences in polar interactions between the drugs and the sorbents of different origins, i.e. differences in content and availability of polar groups in silica surfaces. The participation of endogenous compounds from serum in processes involved in SPE improves the elution and reduces the variations in the elution profiles from serum compared with elution from water, when methanol is the elution liquid. No influence was reported when using acetonitrile as the eluent. The results presented turn the attention to properties of the starting free silica as the main source of the non-standard in SPE of basic drugs.

From the practical point of view, as long as manufacturers will not be able to unify the properties of silica and procedures to obtain its modified forms, work with material of one vendor is the only way how to prevent the manufacturer-to-manufacturer variations.

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