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EFFICIENT AND SELECTIVE ISOLATION OF LOCAL ANAESTHETICS FROM BIOLOGICAL FLUIDS USING C_{18} SILICA GEL

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SUMMARY

A simple and rapid method for the efficient and selective isolation of the local anaesthetics carbisocaine, heptacaine, and pentacaine from serum, saliva or urine has been developed. The method comprises the trapping of substances from the biological fluid by the silica sorbent Silipor C_{18}, followed by washing-out the majority of the retained endogenous compounds with acetonitrile. Finally, the drugs are eluted from Silipor C_{18} with methanol. The advantage of the presented sample clean-up procedure is demonstrated by gas chromatography.

INTRODUCTION

Carbisocaine, heptacaine and pentacaine, derivatives of carbanilic acid (see Fig. 1), are effective local anaesthetics[1] whose pharmacokinetics are being studied at this Institute. A gas–liquid chromatographic (GLC) method for the analysis of these derivatives[2] uses trimethylanilinium hydroxide (TMAH) for on-column alkylation of the thermolabile carbamate group[3]. Although a single-step solvent extraction is fairly efficient for the isolation of these drugs from biological fluids[4], reaction GLC analysis requires clean-up of the samples by a more selective separation process.

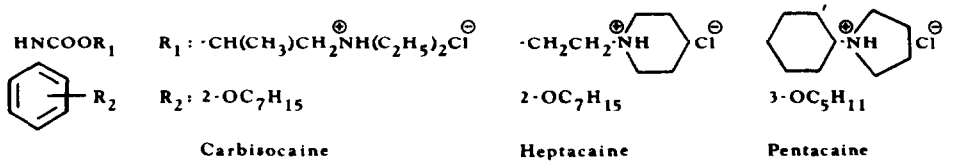

Fig. 1. Chemical structure of carbisocaine, heptacaine and pentacaine.

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In a recent paper[5], the potential for exploiting the different selectivities of common liquids was demonstrated on the pre-separation of pentacaine from aqueous fluids using a Sep-Pak C_{18} cartridge. This paper describes a simple and rapid method for the efficient and selective isolation of carbisocaine, heptacaine and pentacaine from serum, saliva or urine, suitable for the treatment of samples subsequently to be analysed by means of reaction GLC.

EXPERIMENTAL

Materials and chemicals

Carbisocaine, heptacaine and pentacaine were kindly supplied by the Faculty of Pharmacy, Comenius University, Bratislava, Czechoslovakia. [^{14}C]Carbisocaine, [^{14}C]heptacaine, and [^{3}H]pentacaine were synthesized at the Institute for Research, Production and Uses of Radioisotopes, Prague, Czechoslovakia.

Irregularly shaped Silipor C_{18} octadecylsilica gel [16.3% (w/w) C], particle size 125–160 μm, was obtained from the Research Institute of Pure Chemicals, Lachema, Brno, Czechoslovakia.

Acetonitrile, methanol and water were of HPLC grade (J. T. Baker, Phillipsburg, NJ, U.S.A.).

Apparatus and measurements

A JGC-20K gas chromatograph (Jeol, Tokyo, Japan) equipped with a flame-ionization detector was used. Separations were carried out at 260°C in a glass column (1 m × 2 mm I.D.) packed with 3% OV-17 on Chromosorb W (60–80 mesh) (Merck, Darmstadt, F.R.G.). The flow-rate of the carrier gas (nitrogen) was 35 ml/min. Samples (1 μl each) were applied by means of an injection port heated to 280°C.

A Tri-Carb 300 CD liquid scintillation counter (Packard, Downers Grove, IL, U.S.A.) was used for counting the radioactivity. Samples were mixed with SLD 31 dioxane liquid scintillator (Spolana, Neratovice, Czechoslovakia).

Working standards

Aqueous solution I contained 1 μg/μl of carbisocaine and *ca.* 5 nCi/μl of [^{14}C]carbisocaine. Solutions II and III contained heptacaine to [^{14}C]heptacaine and pentacaine to [^{3}H]pentacaine, respectively, in similar concentration ratios as in solution I.

Procedures

Human serum, saliva and urine (1 ml each) doped with 10 μl (*ca.* 10^5 dpm) of solution I, II or III was poured on a Silipor C_{18} bed (3 × 9 mm I.D.). The sorbent (0.1 g), fixed in a 2-ml plastic syringe barrel, was conditioned before use by washing with 2 ml of acetonitrile followed by 2 ml of water. After the sample had passed through the syringe barrel the sorbent layer was washed with 2 ml of water and then 2 ml of acetonitrile. The compounds retained by the sorbent were eluted with 5 ml of methanol. The methanolic effluent was collected for determination of the efficiency of the isolation process.

Drug-free biological fluids (1 ml each) were treated by the above procedure (a set of samples was prepared without the acetonitrile washing step). The methanolic

TABLE I

EFFICIENCY OF THE ISOLATION OF LOCAL ANAESTHETICS FROM BIOLOGICAL FLUIDS

Drug	*Recovery (%)**		
	Serum	*Saliva*	*Urine*
Carbisocaine	101 ± 1.8	99 ± 2.0	98 ± 3.4
Heptacaine	101 ± 1.7	100 ± 1.8	99 ± 3.1
Pentacaine	101 ± 2.0	101 ± 2.3	99 ± 3.3

* Means ± standard deviations from triplicate determinations.

effluent was dried under a stream of nitrogen at 50°C and the residue was dissolved in 20 μl of a methanolic solution of TMAH (0.1 μmol/μl; Serva, Heidelberg, F.R.G.) and analysed by GLC.

RESULTS AND DISCUSSION

The isolation of hydrophobic xenobiotics, their metabolites and lipophilic endogenous compounds from biological fluids using C_{18} silica gel is currently being widely utilized. Several advantages of this treatment of biological samples prior to radioimmuno assay[6], high-performance liquid chromatography (reversed-phase[7,8] and normal-phase[9–11]), thin-layer chromatography on silica[12,13] or gas chromatography[14–17] have been reported. The procedures reported so far involve fractionation of a complex mixture by the reversed-phase liquid chromatographic principle. Passing the biological fluids through the sorbent and washing with an aqueous medium corresponds to the elution of trapped compounds from C_{18} silica with a weak eluent. To elute the retained compounds, a liquid with a strong elution strength is applied. Typical recoveries of this liquid–solid phase partition process are greater than 90% with an accuracy unmatched by liquid–liquid extractions[18]. Moreover, if necessary, selective isolation of virtually all classes of retained compounds can be achieved by adjustment of the appropriate elution strength of the washing and/or eluting mobile phase, using a mixture of a weak and a strong eluent[19].

Recently we studied the elutability of pentacaine from a Sep-Pak C_{18} cartridge with various liquids[5]. Proton acceptor solvents (*e.g.*, methanol) have been demonstrated to be the best eluents, whereas predominantly dipole interactive liquids (*e.g.*, acetonitrile) are unable to elute pentacaine from the cartridge packing[5]. Qualitatively similar results were obtained using Silipor C_{18}. Our experiments showed that under the conditions described above, the sorbent totally retained the studied compounds from water-based matrices, whereas methanol eluted all solutes adsorbed on the active surface of Silipor C_{18}. The addition of the acetonitrile washing step did not influence the recovery of the drug isolated and the reproducibility of the isolation process (Table I). On the other hand, most of the retained endogenous compounds were removed from the sorbent by washing with acetonitrile, as the latter is a strong eluent for them. Consequently, the reaction GLC analysis of the blank biological samples (see Fig. 2), processed by the acetonitrile washing step on Silipor C_{18}, revealed no peaks significantly interfering with those of pentacaine and heptacaine eluted at 9.1 and 4.7 min.

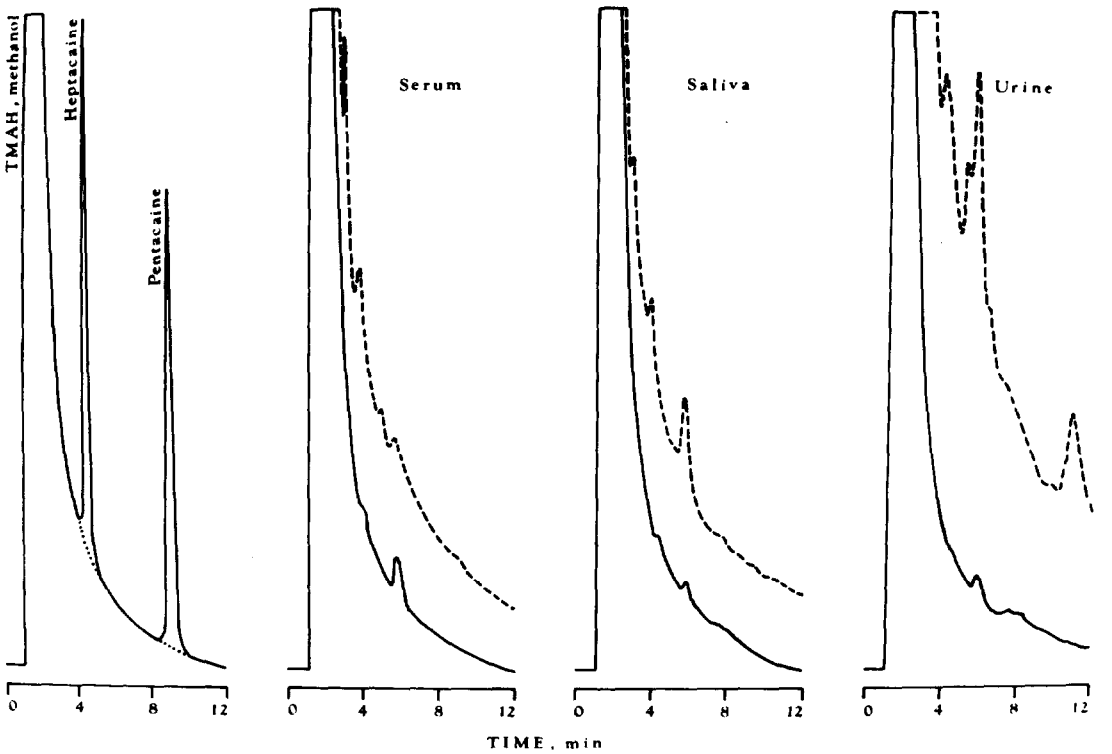

Fig. 2. Chromatograms of the methylated heptacaine, pentacaine and samples obtained from biological fluids treated by using Silipor C_{18} (——) and chromatograms of samples obtained from biological fluids treated without the acetonitrile washing step (——). The concentration of the injected drugs in a methanolic solution of TMAH was 1 μg/μl.

The proposed method is simple, rapid, efficient and sufficiently selective for the treatment of biological samples prior to the determination of local anaesthetics (μg/ml levels and below) by reaction GLC with flame-ionization detection.

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