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Note

Gas chromatographic determination of pentacaine in rat serum

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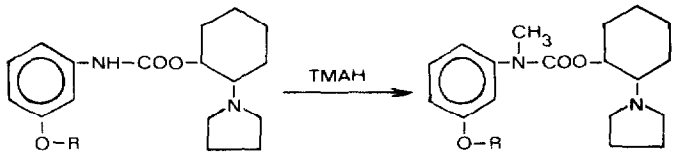
Pentacaine, *trans*-2-(1-pyrrolidino)cyclohexyl-3-pentyloxycarbanilate chloride (Fig. 1), is a local anaesthetic of alkoxycarbanilate type [1] that has recently been undergoing clinical studies. It has been determined in biological fluids only as [^{3}H]pentacaine by selective ion-pair extraction into an organic solvent at low pH [2].



R: C_5H_{11} – PENTACAINE
C_6H_{13}– INTERNAL STANDARD

Fig. 1. Molecular structures of pentacaine, the internal standard and their N-methyl derivatives.

In this paper we describe a gas chromatographic (GC) method based on previous findings of Štefek et al. [3] concerning the possibility of stabilizing the thermally labile carbamate bond in alkoxycarbanilates by its reaction with trimethylanilinium hydroxide (TMAH) in the GC injector (Fig. 1).

For the separation of pentacaine from serum, the method described by Šoltés and co-workers [4, 5] was used to isolate the drug from biological material rapidly and selectively, via its adsorption—desorption properties on C_{18}-silanized silica (Sep-Pak C_{18}, Silipor C_{18}).

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EXPERIMENTAL

Standards and reagents

Pentacaine and *trans*-2-(1-pyrrolidino)cyclohexyl-3-hexyloxycarbanilate chloride (Fig. 1), used as internal standard, were kindly supplied by the Faculty of Pharmacy, Comenius University, Bratislava, Czechoslovakia. Trimethylanilinium hydroxide (0.1 mol l^{-1} in methanol) was purchased from Serva (Heidelberg, F.R.G.). Irregularly shaped Silipor C_{18} silica gel [16.3% (w/w) C], particle size 125—160 μm, was obtained from the Research Institute of Pure Chemicals, Lachema, Brno, Czechoslovakia. The organic solvents (acetonitrile, methanol and benzene, all supplied from Lachema, Brno, Czechoslovakia) were distilled before use as also was the water used for reagents.

Glassware

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

Gas chromatographic conditions

GC separations were performed under isothermal conditions on a Perkin-Elmer 900 gas chromatograph equipped with a flame ionization detector. The glass column (2 m × 2 mm I.D.) was packed with Chromosorb W AW DMCS (80—100 mesh) coated with 2% OV-17. The column temperature was 260° C, injection port temperature 280° C. Nitrogen was used as carrier gas with a flow-rate 40 ml min^{-1}.

Sample preparation

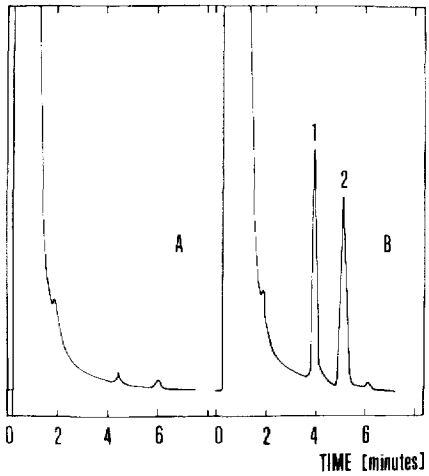
A serum sample (1 ml) was placed in a glass-stoppered tube and 0.1 ml of internal standard solution (10 μg ml^{-1} in water) was added. Silipor C_{18} (100 mg), fixed in a 2-ml plastic syringe barrel [5], was conditioned before use by washing with acetonitrile (1 ml) and water (1 ml), then the serum sample was applied. After the sample had passed through the syringe barrel the sorbent layer was washed with 1 ml of water and then 1 ml of acetonitrile. Pentacaine retained by the sorbent was eluted with 2.5 ml of methanol. The methanol was evaporated to dryness in a water bath at 60° C under a mild stream of nitrogen; 10 μl of TMAH in methanol were added to the dry residue and 1 μl of the resulting solution was injected into the gas chromatograph.

Standard curves

Varying quantitites of pentacaine (0.1—10 μg) were added to 1 ml of rat control serum. After standing overnight, the samples were carried through the analytical procedure. The ratio of the peak height of pentacaine to that of the internal standard was plotted against the concentration of pentacaine.

RESULTS AND DISCUSSION

Typical chromatograms obtained with blank serum before and after spiking with pentacaine and the internal standard are shown in Fig. 2. The peaks of the two compounds were well resolved (R = 2.6) and no interference was observed



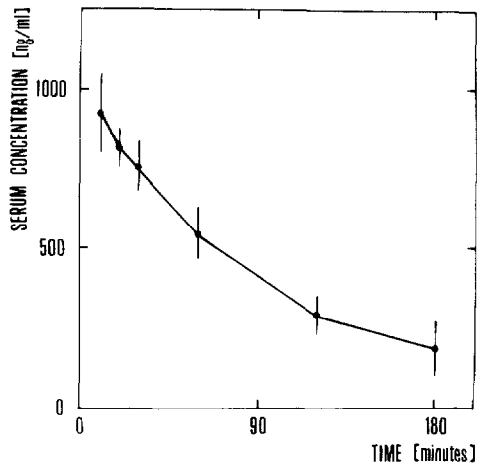


Fig. 2. Gas chromatograms of serum extracts. (A) Drug-free serum; (B) serum spiked with pentacaine (1) and the internal standard (2), both in a concentration of 1 μg/ml.

Fig. 3. Serum concentration—time curve of pentacaine after intravenous administration of 4 mg/kg to rats (n = 6).

TABLE I

REPEATABILITY AND RECOVERY OF THE METHOD AS APPLIED TO SPIKED RAT SERUM SAMPLES

Amount added (μg ml^{-1})	Average of six assays ± S.D. (μg ml^{-1})	R.S.D. (%)
0.1	0.11 ± 0.01	9.1
0.5	0.52 ± 0.03	5.8
1.0	0.96 ± 0.08	8.3
5.0	5.18 ± 0.10	1.9
10.0	9.92 ± 0.11	1.1

from endogenous substances. Interference from metabolites was not anticipated because of their different chromatographic behaviour [6].

The absolute recovery of the extraction procedure determined with ^{3}H-labelled pentacaine was found to be 83.4%. A standard calibration curve obtained after extraction of pentacaine from serum was linear in the concentration range studied ($y = 0.741x + 0.021$; $r = 0.9995$). The repeatability and relative recovery of the method are reported in Table I.

The absolute sensitivity of the flame ionization detector for pentacaine (10 ng) allowed application of the method for the determination of serum levels of pentacaine in rats after a single intravenous administration of the drug. Fig. 3 illustrates these levels up to 3 h post dose.

The serum levels determined by the described GC method are in good agreement with the results of Bezek et al. [7], which were obtained with ^{3}H-labelled pentacaine.

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