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Note

Capillary gas chromatographic method for the simultaneous determination of local anaesthetics in plasma samples

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Local anaesthetics are drugs that reversibly block the conduction of impulses in the peripheral nervous system. They vary in their clinical profiles, and these differences may be related to their chemical structures [1,2]. The amide types are widely used for various local or regional treatments. The dose administered and the pharmacokinetic profile of the drug determine the concentration and the sensitivity required for the analytical method. Determination of the free fraction of local anaesthetics, especially for those which are extensively protein-bound, requires highly sensitive methods.

A great number of gas or liquid chromatographic assays for the determination of single local anaesthetics in biological samples have been published (*e.g.* refs. 3–11). However, the limit of quantification for most of the published methods is not sufficient for the determination of the free fraction or for the calculation of pharmacokinetic parameters. In routine analyses of a great number of samples, where automatization may be of interest, it is advantageous to use the same analytical method to determine plasma concentrations of various kinds of local anaesthetics.

This paper describes a sensitive and simple capillary gas chromatographic (GC) method for the determination of five local anaesthetics (Fig. 1) in plasma samples. The simplicity of the described method makes it well suited for automatization. The method can also be used for the determination of several anaesthetics, if they are administered simultaneously, and as a simple means of identifying local anaesthetics by their retention times.

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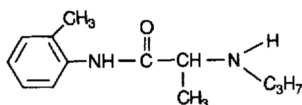

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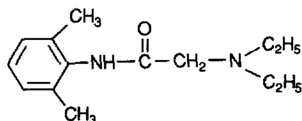

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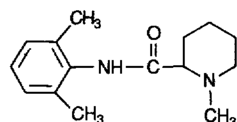

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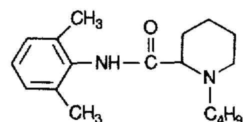
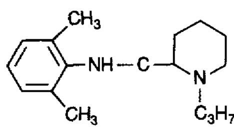
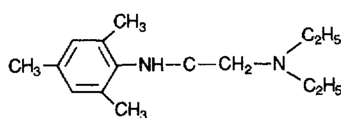


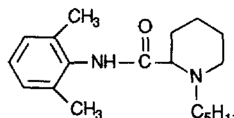
Bupivacaine IV



Ropivacaine V



Mesocaine VI



Pentycaine VII

Fig 1 Structures of compounds I–VII Ropivacaine (V) is a new long-acting local anaesthetic under investigation Mesocaine (VI) and pentycaine (VII) are the internal standards

EXPERIMENTAL

Chemicals

Prilocaine (I), lidocaine (II), mepivacaine (III), bupivacaine (IV) and ropivacaine (V) were obtained, as hydrochloride salts, from Astra Pharmaceutical Production (Södertälje, Sweden). The internal standards, mesocaine (VI) and pentycaine (VII), were synthesizede at Astra Pain Control (Södertälje, Sweden). Other chemicals were of analytical-purity grade.

Gas chromatography

The gas chromatograph was a Hewlett Packard 5890 (Hewlett Packard, Palo Alto, CA, U.S.A.) equipped with a nitrogen–phosphorus detector and an open tubular column (Hewlett Packard cross-linked methyl silicone, 17 m × 0.2 mm

I.D., film thickness 0.33 μm). Splitless injections were performed with a Hewlett Packard 7672A automatic sampler, and the chromatograms were recorded and analysed with P. E. Nelson Access*Chrom GC/LC data system (Perkin Elmer Nelson Systems, Cupertino, CA, U.S.A.).

The carrier gas (helium) flow-rate was 0.5 ml/min which corresponds to a pressure of 117 kPa. Hydrogen and air flow-rates in the detector were 3–4 and 100–120 ml/min, respectively. Injector and detector temperatures were 260°C. The oven temperature was kept at 150°C for 1 min followed by a 40°C/min programme. The final temperature, 240°C, was kept for 6.8 min

Sample preparation

To each tube containing 1 ml of plasma sample was added 0.5 ml of an aqueous solution containing 3 μmol/l internal standard (mesocaine was used for the determination of prilocaine and lidocaine, and pentycaine for mepivacaine, ropivacaine and bupivacaine) followed by 500 μl of a 10% (w/v) aqueous solution of sodium carbonate and 4 ml of *n*-hexane–methylene chloride (4:1, v/v). After gentle agitation of the tubes in a rotating mixer for 20 min and centrifugation for 10 min at 3200 *g*, the organic layer was transferred to another tube and evaporated to dryness at 40°C under a stream of nitrogen. The residue was reconstituted in 200 μl of an *n*-hexane–ethanol (9:1) mixture.

Plasma samples were stored in polypropylene tubes at −20°C until analysis.

A 3-μl aliquot was injected into the chromatograph. The splitless valve time was 0.5 min.

Quantitation

Standard samples were prepared by adding known amounts of the drug to 1-ml aliquots of pooled human plasma. The peak-height ratio of the drug to the internal standard was plotted against the concentration of the drug. The linear range was initially established from a number of conventional calibration curves.

Quantitations were, thereafter, based on injection of six plasma standard samples with the same concentration. The concentration of the standard samples was chosen in relevance to the level of the unknown samples, normally 1 μmol/l. Blank plasma was also analysed on each occasion to confirm the origin of the calibration graph. In order to check the performance of the analytical system, control samples with known concentrations of the drugs were analysed together with the unknown samples. Two levels were used, *i.e* three times higher and ten times lower.

RESULTS AND DISCUSSION

The recovery of the extraction procedure was obtained from the slopes of two calibration curves. The plasma curve was prepared from twenty spiked plasma samples processed as described in the Experimental section (but without internal

TABLE I

INTER-ASSAY PRECISION AND ACCURACY FOR CONTROL SAMPLES

Drug	Level (nmol/l)	Inter-assay precision (C V , %)	Accuracy (% of added amount)	*n*
Prilocaine	200	6 9	96	8
	3800	2 6	100	8
Lidocaine	200	3 2	104	8
	2900	1 5	98	8
Mepivacaine	250	4 7	100	8
	1200	3 4	100	8
Ropivacaine	34	13 8	111	14
	1100	7 9	102	13
Bupivacaine	25	11 7	108	7
	1800	4 6	100	8

standard and using exact volumes) while the reference curve was prepared from unextracted ethanol solutions. The internal standard was added in the reconstitution step. The absolute recovery was found to be 79, 85, 103, 92 and 89% for prilocaine, lidocaine, mepivacaine, ropivacaine and bupivacaine, respectively.

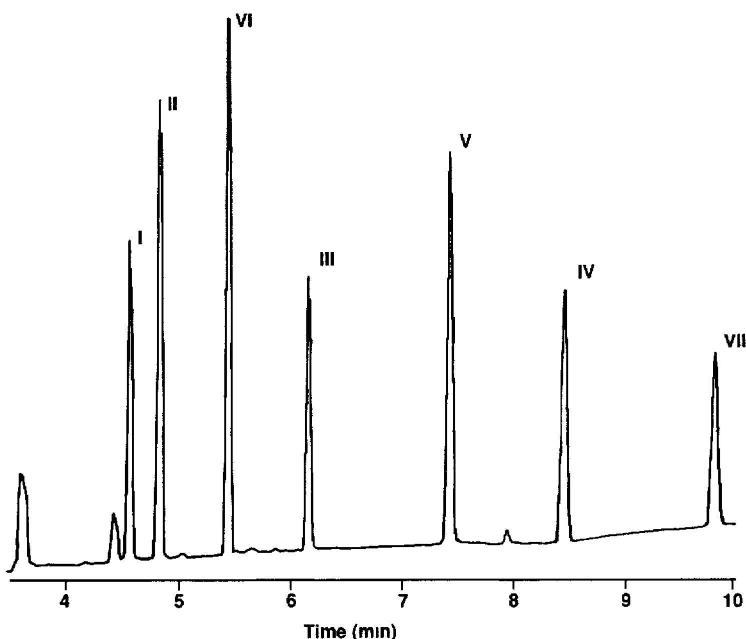


Fig 2 Chromatogram from human plasma spiked with prilocaine (I, 1 7 μmol/l), lidocaine (II, 2 1 μmol/l), mesocaine (VI, 2 6 μmol/l), mepivacaine (III, 1 4 μmol/l), ropivacaine (V, 1 6 μmol/l), bupivacaine (IV, 1 3 μmol/l) and pentycaine (VII, 1 1 μmol/l)

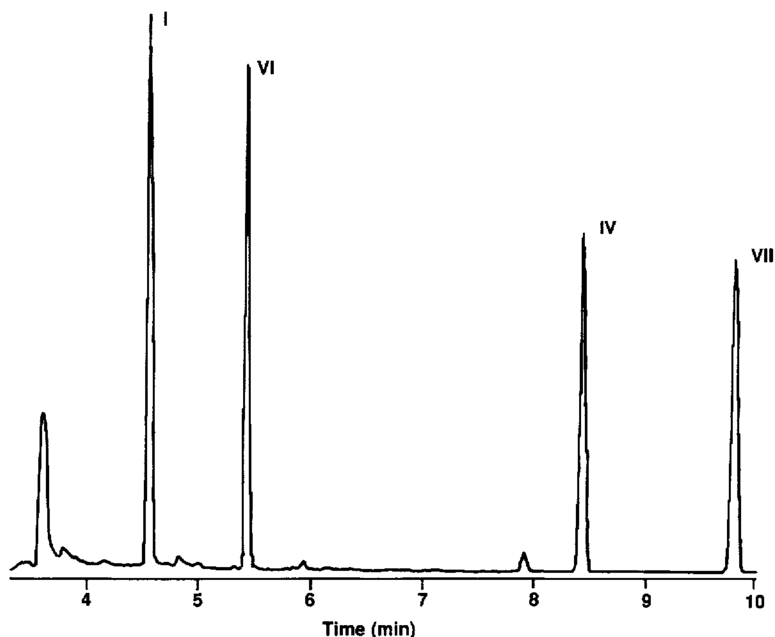


Fig 3 Chromatogram of dog plasma The sample was taken 1 h after single subcutaneous administration of 78 μmol/kg prilocaine and 12 μmol/kg bupivacaine Peaks I = prilocaine (4 0 μmol/l), VI = mesocaine, IV = bupivacaine (2 2 μmol/l); VII = pentycaine

Limits of quantification were set at 40 nmol/l. At this level, endogenous plasma components did not interfere with the local anaesthetics, and the coefficients of variation (C.V.) were approximately 10%. The calibration curves were found to be linear up to 3 μmol/l.

The inter-assay precision and accuracy, shown in Table I, were calculated from control samples which were analysed in duplicate together with unknown samples on different occasions. The standard deviation was calculated from the mean of each duplicate

The plasma samples of local anaesthetics could be determined with an inter-assay precision of 1.5–10%, and the accuracy was close to 100%.

A chromatogram obtained from human plasma spiked with all local anaesthetics (Fig. 2) illustrates the selectivity of the described method. The method has, in addition to numerous analyses of samples containing a single local anaesthetic, been successfully used to analyse samples from studies at Astra when a simultaneous administration of prilocaine and bupivacaine was investigated. Fig. 3 shows a chromatogram of a dog plasma sample after subcutaneous administration.

CONCLUSION

The present method, using single-step extraction and automated capillary column chromatography with nitrogen-selective detection, allows rapid and reproducible quantification. The sensitivity is sufficient for the determination of free fractions and calculation of pharmacokinetic parameters. In routine analyses, 30 determinations can be performed per day by one person. At our laboratory about 10 000 plasma concentrations of the various local anaesthetics have been determined during the last three years using the described method.

ACKNOWLEDGEMENTS

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