Journal of Pharmaceutical & Biomedical Analysis
Vol. 4, No. 3, pp. 333–340, 1986
Printed in Great Britain

0731–7085/86 $3.00 + 0.00
Pergamon Journals Ltd.

Determination of carbisocaine, heptacaine and pentacaine in plasma by capillary gas chromatography with nitrogen-selective detection

V. MARKO,*† J. WIJSBEEK and R. A. DE ZEEUW

Department of Analytical Chemistry and Toxicology, State University, Deusinglaan 2, 9713 AW Groningen, The Netherlands

Abstract: A gas–liquid chromatographic method is described for the determination of the local anaesthetics carbisocaine, heptacaine and pentacaine in plasma. A C_{18} solid-phase extraction was used in a modification to increase selectivity. Following on-column derivatization with trimethylanilinium hydroxide, the analytes were determined by means of capillary gas chromatography and nitrogen–phosphorus selective detection. In comparison with flame ionization detection, the sensitivity of NPD was 20 times higher with a limit of determination in plasma of 10 ng ml^{-1}.

Keywords: *Capillary gas chromatography; on-column methylation; solid-phase extraction; assay of local anaesthetics.*

Introduction

Carbisocaine, *N*-[2-(2-heptyloxyphenylcarbamoyloxy)-2-methylethyl]diethylammonium chloride, heptacaine, *N*-[2-(2-heptyloxyphenylcarbamoyloxy)ethyl]piperidinium chloride, and pentacaine, *N*-[*trans*-2-(3-pentyloxyphenylcarbamoyloxy)cyclohexyl]pyrrolidinium chloride (Fig. 1) are local anaesthetics (LA) of the alkoxycarbanilate type, with high infiltrate as well as topical anaesthetic activities [1], that are currently being evaluated.

Pharmacokinetic studies in animals using the radiolabelled compounds, [^{14}C]-carbisocaine, [^{14}C]-heptacaine and [^{3}H]-pentacaine, have shown these drugs to have large distribution volumes and as a result low concentrations in blood [2–4]. Therefore a sensitive analytical method is needed to conduct detailed pre-clinical and clinical pharmacodynamic and pharmacokinetic studies.

A gas chromatographic method has been described for the determination of pentacaine in serum [5]. Flash-heater methylation of the drug with trimethylanilinium hydroxide was employed along with gas chromatography on packed columns with flame ionization detection. However, the method is of limited sensitivity and is inappropriate for full-range pharmacokinetic studies.

*To whom correspondence should be addressed.
†Permanent address: Institute of Experimental Pharmacology, Centre of Physiological Sciences, Slovak Academy of Sciences, 842 16 Bratislava, Czechoslovakia.

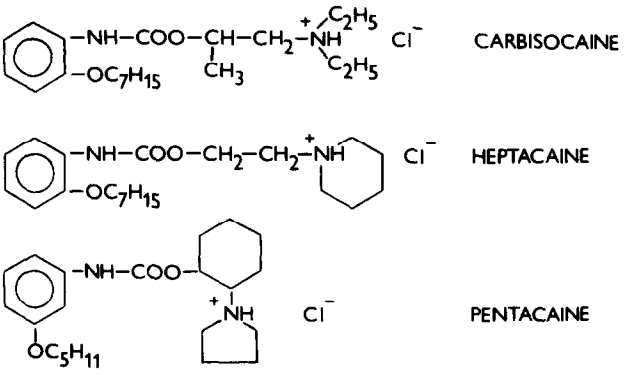

Figure 1
Molecular structures of the local anaesthetics studied.

A highly sensitive and selective capillary gas chromatographic method using nitrogen-specific detection is now described for the determination of carbisocaine, heptacaine and pentacaine in plasma. The method retains the extraction and derivatization procedures of the earlier work [5].

Experimental

Gas chromatography

A Hewlett–Packard Model 5880A gas chromatograph was used in conjunction with a 5880A level four terminal and a Model 7671 Hewlett–Packard autosampler. The chromatograph was equipped with thermionic selective nitrogen (NPD) and flame ionization (FID) detectors.

A fused silica capillary column CP-Sil 5 (25 m × 0.32 mm, film thickness 0.48 μm) (Chrompack, Middelburg, The Netherlands) was used.

The gas chromatographic separations were achieved under temperature programmed conditions using the following sequence: initial temperature 60°C for 2.5 min; program at 20°C/min to 200°C and hold for 0.01 min; then program at 10°C/min to 315°C and hold for 1.0 min.

The temperatures of the splitless injection port and detectors were 300°C and 315°C, respectively. Helium used as carrier gas was maintained at a linear velocity of 45 cm s^{-1}. The purge activation time was 50 s.

Reagents and chemicals

Carbisocaine, heptacaine and pentacaine were synthesized in the Faculty of Pharmacy of Comenius University (Bratislava, Czechoslovakia). *N,N,N*-trimethylanilinium hydroxide (TMAH, 0.1 M in methanol) was purchased from Serva (Heidelberg, FRG).

CP-Elut C18 extraction columns (Chrompack, Middelburg, The Netherlands), packed with 200 mg of sorbent, were used in the extraction step.

Analytical grade acetonitrile, ethyl acetate and methanol were obtained from Merck (Darmstadt, FRG).

Extraction procedure

The solid-phase extraction method described by Šoltés *et al.* [6] was used for the rapid and selective isolation of the analytes from biological materials.

The extraction columns were conditioned before use by washing with 2 ml of acetonitrile and then 2 ml of water. Blank calf plasma or samples (2 ml) spiked with 10 ng ml^{-1} to 10 μg ml^{-1} of carbisocaine, heptacaine and pentacaine were then introduced. After passage of the samples, the extraction columns were washed with 2 ml of water and then 2 ml of acetonitrile to elute the majority of hydrophilic and hydrophobic endogeneous compounds. The analytes which remain in the extraction column are then eluted with 1 ml of methanol. The methanol was evaporated to dryness at 60°C under a gentle stream of nitrogen. Ethyl acetate (80 μl) and TMAH in methanol (20 μl) were added to the residue and 3 μl of this solution was injected into the gas chromatograph. When measuring the two highest concentrations of local anaesthetics, namely 5 and 10 μl ml^{-1}, 1 ml of plasma was used with 60 μl of ethyl acetate and 40 μl of the TMAH solution in methanol being added.

Derivatization

The 'on-column' formation of the *N*-methyl derivatives of carbisocaine, heptacaine and pentacaine (Fig. 2) was studied, using 0.25 mM solutions in ethyl acetate and 5, 25, 50 and 100 mM solutions of TMAH in methanol. Volumes of 70 or 80 μl of ethyl acetate solution and 30 or 20 μl of TMAH in methanol were placed into 100 μl crimp-top vials to give 100 μl of mixture with excess concentrations of TMAH against analyte in the range 5–170. These mixtures were chromatographed according to the described procedure using 3-μl injection volumes.

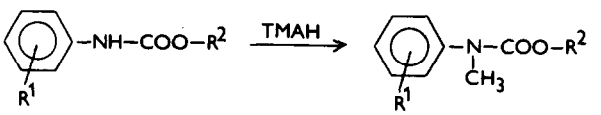

Figure 2
Methylation of the nitrogen of the carbanilate group by trimethylanilinium hydroxide (TMAH).

Evaluation of calibration curve

The response of the detectors were studied over a concentration range of three to four orders of magnitude, starting at 10 ng ml^{-1}. For this reason, the curves presented here are transformed to a logarithmic scale to avoid loss of information, particularly in the lowest concentration range. Calculations were carried out without this transformation.

Results and Discussion

Extraction

Three methods for the isolation of carbisocaine heptacaine and pentacaine from biological material have been described recently: namely, ion-pair liquid–liquid extraction from a strongly acidic solution with benzene [7], normal liquid–liquid extraction from a basic solution with *n*-heptane [8, 9] and liquid–solid isolation using C_{18}-silica gel [6]. The first two methods have been used mostly for the determination of the labelled forms of these drugs [2–4]. The third method has been shown to be

sufficiently fast, efficient and selective for chromatographic purposes [5, 10]. The elution of the extraction column with acetonitrile, after passage of the sample has no effect on the adsorption of the analytes. This is manifested by the observed 95% recovery by this separation procedure (at the concentration of 100 ng ml^{-1} of plasma). On the other hand, there is evidence of the pre-treatment affecting the selectivity of isolation [10].

Derivatization

The main problem encountered during the gas-chromatographic determination of local anaesthetics of the alkoxycarbanilate type arises from their thermal instability. On injection molecular cleavage occurs to yield the corresponding isocyanates and alcohols [11]. The obvious need to stabilize the carbamate bond in these molecules has led to a study of the reactivity of this bond towards various derivatization reagents. As a result it was found that flash-heater methylation with trimethylanilinium hydroxide was a suitable method of stabilization [12]. The methylation is simple, reproducible and proceeds quantitatively, enabling the rapid gas chromatographic analysis of the resulting compounds without decomposition.

A molar ratio of at least 5:1 between TMAH and the analytes in the solution injected into the gas chromatograph was found to be necessary for quantitative methylation with packed columns [5, 12]. In contrast, as shown in Fig. 3, this ratio is insufficient with capillary columns splitless-mode injection, although the injection port temperature was 20°C above that used with packed column separation. The large difference needed between the molar excess of TMAH in packed column and capillary column GLC, namely 5:1 against 100:1, may result from the different column temperatures employed during the separation. In the case of the packed column, the gas chromatographic separations were performed under isothermal conditions at a column temperature of

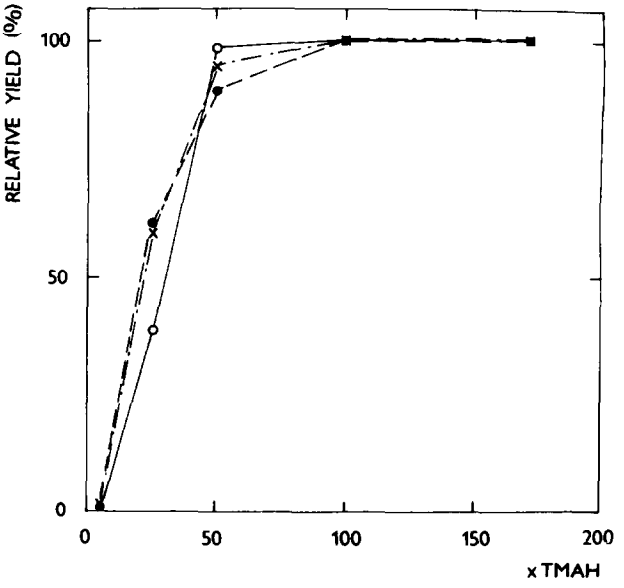

Figure 3
The effect of TMAH concentration on the rate of formation of the *N*-methyl derivatives of carbisocaine (○), heptacaine (×) and pentacaine (●).

260°C. On the other hand, in the capillary column mode, the initial temperature was 60°C which was held for 2.5 min, and only after this time was the temperature elevated. It is possible that, with the packed column separations, part of the derivatization reaction takes place at the top of the column.

Chromatography

The applicability of the capillary GLC-NPD method to the determination of the alkoxycarbanilate local anaesthetics was examined in experiments on plasma spiked with known amounts of carbisocaine, heptacaine and pentacaine. In order to illustrate the advantages of using the NPD in the present context a non-selective detector, namely the FID, was used and the differences in the responses of these two detectors investigated with respect to their sensitivity towards the local anaesthetics.

Chromatograms shown in Figs 4 and 5 show large peaks between 4 and 8 min, which are due to excess derivatization reagent. These peaks are obtained using both detectors, but this is the only similarity between them. As can be seen, the detectors differ in respect to both sensitivity and selectivity, as well as in the linearity of their responses towards the drugs studied.

The first two differences are clearly represented by Figs 4 and 5. A high 'noise' with blank plasma, together with lower analyte peak heights in the case of the flame ionization detector resulting in a minimal detectable concentration of about 100 ng per ml of plasma. This concentration is the same as when using the FID together with packed column separation [5].

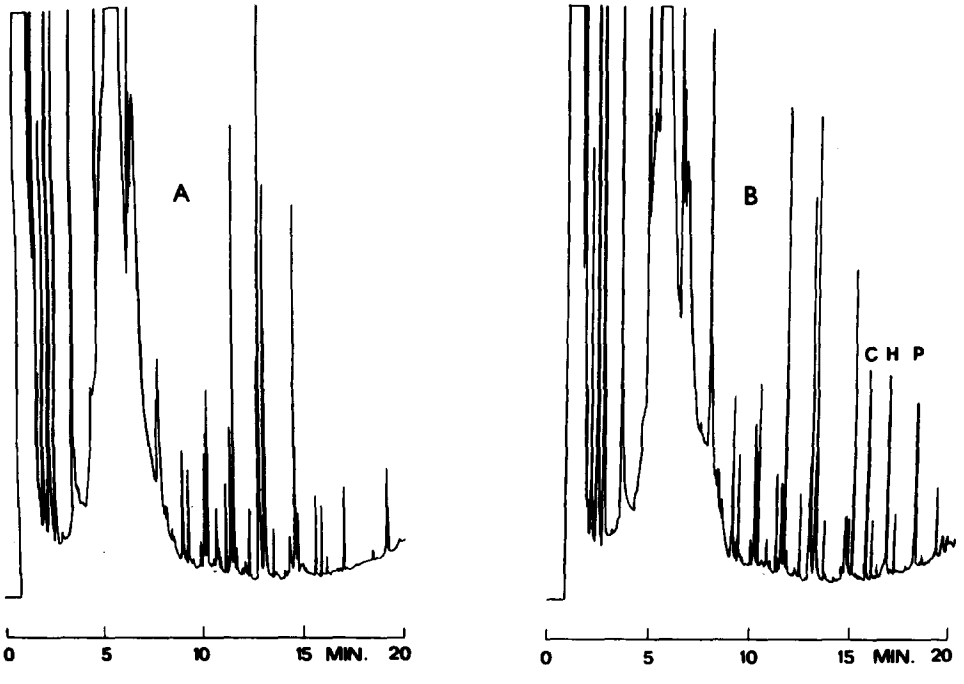

Figure 4
Gas chromatograms of plasma extracts obtained with flame ionization detection (A) Plasma blank; (B) plasma spiked with 500 ng ml^{-1} of carbisocaine (C), heptacaine (H) and pentacaine (P), respectively.

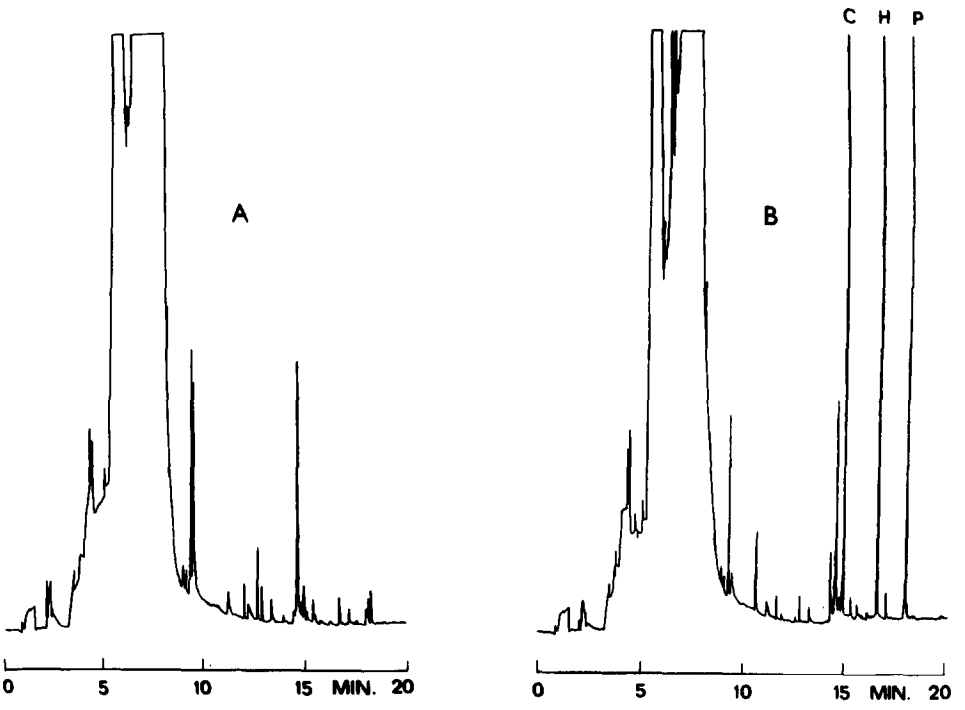

Figure 5
Gas chromatograms of plasma extracts obtained with specific nitrogen detection. (A) Plasma blank; (B) plasma spiked with 500 ng ml^{-1} of carbisocaine (C), heptacaine (H) and pentacaine (P), respectively.

On the other hand, the chromatograms after nitrogen selective detection are 'cleaner' and achieved responses which are approximately 20 times higher.

The calibration graphs for carbisocaine and pentacaine added to plasma (heptacaine was used as an internal standard) are illustrated in Figs 6 and 7 (on a logarithmic scale). They reveal a linearity of response only with the FID. Using this detector, the equations for the calibrations are as follows: carbisocaine, $y = 1.001x + 0.151$; and pentacaine, $y = 0.994x - 0.134$. The correlation coefficients in both cases are equal to 0.999.

Using the specific detector, only dependences within one order of the concentration ratios can be considered to be approximately linear.

The deviation from linearity with the high concentration ratios was not observed for the same samples with the flame ionization detector (Fig. 6). This suggests that the deviation is caused by the non-linear response of the nitrogen-selective detector itself. The deviation is similar for both local anaesthetics and it can be compensated by the internal standard.

Quite a different situation exists in the case of the apparent low concentration non-linearities in the NPD-response. Firstly, the degree of the deviation was in this case dependent upon the compound studied. Further, while in the high-concentration non-linearities the curves for both analytes were lower than the values expected, in the low-concentration ratios the apparent concentrations of carbisocaine were higher than expected whilst those of pentacaine were lower. These differences indicate an unequal behaviour of carbisocaine, heptacaine and pentacaine during their determination, which, however, does not appreciably affect the acceptable repeatability of the determination (see Table 1).

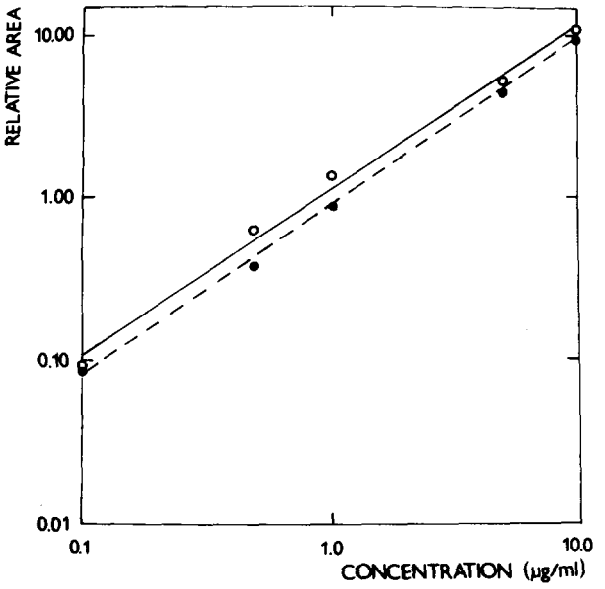

Figure 6
Calibration graphs for carbisocaine (○) and pentacaine (●) after on-column methylation and gas chromatography with flame ionization detection (heptacaine as an internal standard at a concentration of 1 μg ml^{-1}).

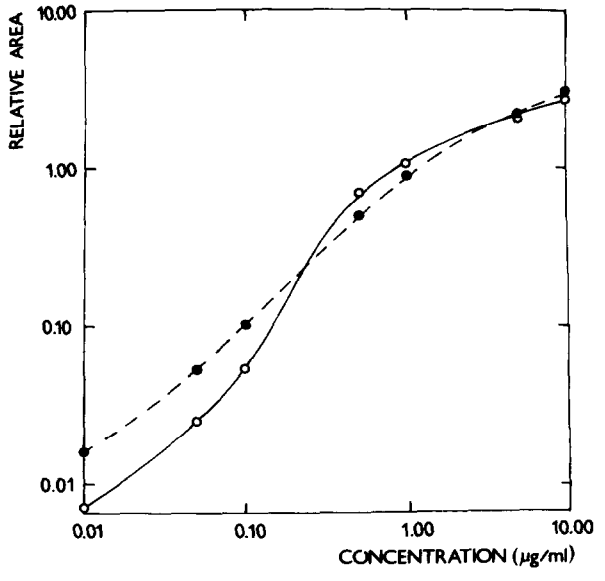

Figure 7
Calibration graphs for carbisocaine (○) and pentacaine (●) after on-column methylation and gas chromatography with nitrogen selective detection (heptacaine as an internal standard at a concentration of 1 μg ml^{-1}).

Table 1
Reproducibility of the determination of two concentrations (10 and 100 ng ml^{-1}) of carbisocaine and pentacaine in plasma using flame ionization detection (FID) and the nitrogen–phosphorus detection (NPD)

	Relative standard deviation*			
	Carbisocaine†		Pentacaine†	
Detector	10 ng ml^{-1}	100 ng ml^{-1}	10 ng ml^{-1}	100 ng ml^{-1}
FID	—	5.3	—	9.1
NPD	9.5	9.0	8.7	9.0

*n = 5.
†Concentration of an internal standard (heptacaine) = 1 μg ml.

In conclusion, the comparison of the flame ionization detector and the nitrogen-selective detector in the determination of carbisocaine, heptacaine and pentacaine showed that better sensitivity and better selectivity was obtainable with the latter whilst the repeatability was similar (see Table 1). The increased sensitivity of the NPD revealed the loss of the linearity of the determination in the low concentration area, which was not noticed previously [5]. The results indicate that capillary gas–liquid chromatography combined with NPD is capable of determinations at the levels found in plasma samples in pharmacokinetic studies; certainly down to concentrations of 10 ng ml^{-1}. For comparison, the values of plasma concentrations of carbisocaine, heptacaine and pentacaine in rats, estimated from their pharmacokinetic parameters, are within the range of tens of nanograms per milliliter 24 h after i.v. administration [2–4].

Acknowledgements: This work was supported by a grant given to V.M. in 1984 by the European Science Foundation, Programme of Grants for Training and Research in Toxicology.

References

[1] S. Štolc and T. Stankovičová, *Drugs Exp. Clin. Res.* in press.
[2] Š. Bezek, V. Ščasnár, T. Trnovec, M. Ďurišová, V. Faberová and L. Beneš, *Biopharm. Drug Disp.* in press.
[3] V. Ščasnár, Z. Kállay, Š. Bezek, T. Trnovec and M. Ďurišová, *Abstr. Int. Symp. Pharmacol. Stabil. Excitable Membranes*, Smolenice, p. 63 (1984).
[4] T. Trnovec, V. Faberová, V. Ščasnár, M. Ďurišová. Š. Bezek and Z. Kállay, *Abstr. Int. Symp. Pharmacol. Stabil. Excitable Membranes*, Smolenice, p. 68 (1984).
[5] V. Marko, M. Štefek and L. Šoltés, *J. Chromatogr.* **339**, 410–413 (1985).
[6] L. Šoltés, L. Beneš and D. Berek, *Meth. Find. Exptl. Clin. Pharmacol.* **5**, 461–465 (1983).
[7] V. Ščasnár, L. Beneš, S. Bezek and T. Trnovec, *J. Radioanal. Nucl. Chem.* **82**, 287–297 (1984).
[8] V. Ščasnár, L. Beneš, Š. Bezek and T. Trnovec, *Radiochem. Radioanal. Lett.* **59**, 45–52 (1983).
[9] V. Ščasnár, L. Beneš, Š. Bezek and T. Trnovec, *Pharmazie* **40**, 268–269 (1985).
[10] L. Šoltés, D. Berek and M. Štefek, *J. Chromatogr.* **286**, 223–227 (1984).
[11] M. Štefek, L. Beneš and V. Kováčik, *Chem. Zvesti* **37**, 209–212 (1983).
[12] M. Štefek, L. Beneš and V. Kováčik, *Chem. Zvesti* **37**, 213–216 (1983).

[*Received for review 18 April 1985; revised manuscript received 25 July 1985*]