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Note

Detection of some local anaesthetics in horse urine and plasma by gas–liquid chromatography

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Several analytical methods for the determination in biological materials of local anaesthetics alone or in combination with their metabolites have been published[1–10]. Although enzyme immunoassay[1] and high-performance liquid chromatography[2] have been employed for the determination of lidocaine, gas–liquid chromatography (GLC) is currently the method most widely used for measuring local anaesthetics[3–10]. The use of the nitrogen specific detector in the analysis of local anaesthetics has mainly been limited to lidocaine[3,11,12] and etidocaine[12], while electron capture detection after derivatization with heptafluorobutyric acid anhydride has been proposed for the determination of procaine in horse plasma[13].

In the context of doping analysis in race horses, few studies have been undertaken of the detection of a series of local anaesthetics in horse urine and plasma. Nevertheless, several of these compounds appear on the list published by the Association of Official Racing Chemists (A.O.R.C.) as positive results reported by their members during the period 1949–1976.

Although a lot of work has been done on the detection of procaine[13,14] which has both local anaesthetic and central stimulant actions in the horse[14,15], only one gas chromatography–mass spectrometry (GC–MS) study on a series of local anaesthetics has been published[16]. Since most laboratories do not currently use a GC–MS apparatus in routine analysis of doping substances in race horses, we have developed a GLC method using a nitrogen specific detector for the screening of some local anaesthetics in small volumes of horse plasma and urine.

EXPERIMENTAL

Materials

The following local anaesthetics were studied: Amylocaine hydrochloride (Rhone Poulenc, Paris, France), Butanilicaine triphosphate (Hoechst, Frankfurt/M, G.F.R.), Etidocaine hydrochloride (Astra, Södertälje, Sweden), Lidocaine hydrochloride (Astra), Mepivacaine hydrochloride (Astra), Oxybuprocaine hydrochloride (Sandoz, Basle, Switzerland), Pramocaine hydrochloride (Abbott, North Chicago, IL, U.S.A.), Prilocaine hydrochloride (Astra), Procaine hydrochloride (E. Merck, Darmstadt, G.F.R.), Tetracaine hydrochloride (Bayer, Leverkusen, G.F.R.) and Trimecaine hydrochloride (Astra). Echothiophate iodide was kindly donated by Ayerst (New York, NY, U.S.A.).

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All glassware was silanized as described previously[17], and the organic solvents (analytical grade) were freshly distilled before use.

Instrumental conditions

A Varian Model 1400 gas chromatograph equipped with a flame ionization detector and connected to a Varian CDS 111 Integrator was used for the determination of Kováts' indices. The glass columns (2 m) used were: 5% OV-101 (A), 3% OV-7 (B) and 3% OV-25 (C) on Chromosorb W (80–100 mesh).

A Varian Model 3700 gas chromatograph equipped with nitrogen specific and ^{63}Ni detectors and connected to a Varian CDS 111 Integrator was used for the determination of the extraction recoveries. All analyses were performed on column B.

Dilutions were made with a Hamilton digital diluter/dispenser.

Procedure

Kováts' indices. Solutions containing the local anaesthetic (1 mg/ml) were made in methanol. A 1-μl volume was injected together with the appropriate *n*-alkanes dissolved in *n*-hexane. The retention times were measured using the integrator and each analysis was done in quadruplicate for each column.

Extraction recovery. Blank horse urine (2 ml) or blank horse plasma (2 ml) to which were added 100 μl of Echothiophate iodide (1 mg/ml in water) were spiked respectively with 125, 50 and 25 μl of an aqueous solution of the local anaesthetic (10 ng/μl), followed by 200 μl of NH_4^+/NH_3 buffer (pH 9.4). After extraction with 6 ml redistilled cyclohexane (5 min) and centrifugation (5 min), 5 ml of the organic layer were transferred to a clean silanized conical tube and evaporated to dryness under nitrogen (40°C). The residue was redissolved in 50 μl of an appropriate methanolic standard solution (20 or 40 ng/μl) of a closely related local anaesthetic or analogue and 1 μl injected. The whole procedure was repeated six times for each concentration.

This procedure was repeated with two extraction steps using 5 ml cyclohexane. The combined organic layers (4 and 5 ml) were evaporated and treated as above. A calibration graph was constructed and least-squares linear regression analysis performed using different concentrations of the local anaesthetic and the appropriate internal standard dissolved in methanol (Fig. 1).

Due to non-linear adsorption and the resulting excessive peak tailing, the extraction recovery for Oxybuprocaine was measured after derivatization. Therefore, the residue obtained after the evaporation of the cyclohexane phase was redissolved in 1 ml internal standard solution (1 ng/μl Tetracaine hydrochloride in chloroform), treated with 20 μl trifluoroacetic acid anhydride and 5 μl pyridine–benzene (5:100) and allowed to react at room temperature during 15 min. After evaporation to dryness under nitrogen (40°C) the residue was redissolved in 50 μl ethyl acetate and washed with 20 μl 0.01 *N* NaOH; 1 μl of the organic layer was injected into the gas chromatograph (column B, 215°C). A calibration graph was constructed in an appropriate manner.

The extraction recovery for Butanilicaine was determined after reaction with pentafluorobenzoyl chloride and will be described elsewhere.

Routine detection limit. Different amounts (10–50 μl) of the local anaesthetic solution (0.5 ng/μl) were added to 2 ml horse plasma or urine. The extraction was

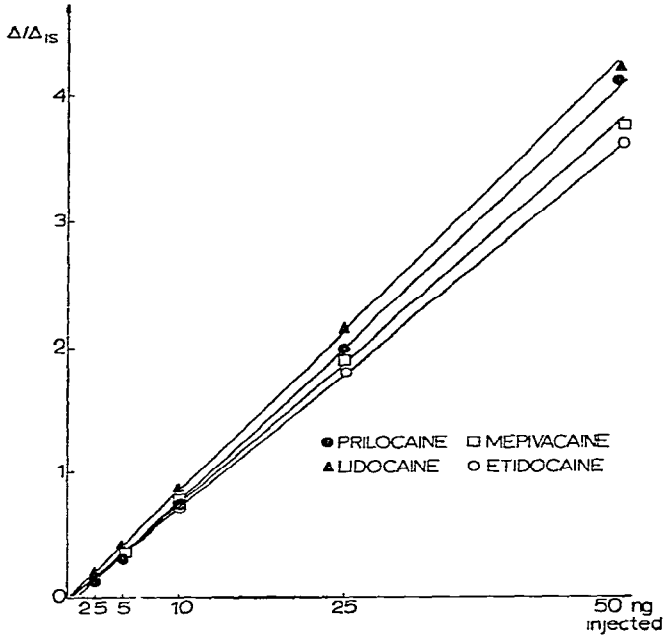

Fig. 1. Calibration graph for Prilocaine, Lidocaine, Mepivacaine and Etidocaine. Internal standard: 2-chloro-6-methylphenylacetamide, 40 ng/μl.

performed (two extraction steps) as above, the residue redissolved in 20 μl methanol and 1 μl was injected. Non-spiked plasma or urine was analysed in the same manner and routine detection limits determined based on a peak-to-noise ratio of 4:1. Maximum detection sensitivity was obtained by carefully adjusting bead current, and hydrogen and air flow-rates.

RESULTS AND DISCUSSION

Since the retention index, which is specific for each substance, is mainly dependent on the column material and oven temperature[18], the differences between the retention indices for one compound on two or more stationary phases are characteristic of that compound. The use of Kováts' indices and inter-column retention differences for standardization in GC enables the compilation of GC data and their exchange between different laboratories. For these reasons the Kováts' indices and inter-column retention differences of some local anaesthetics on three stationary phases are given in Tables I and II. The measurements were done at two different temperatures for each compound. Since these physical measurements should be done very carefully[19,20], the retention times of the different peaks were measured electronically while parameters such as the carrier gas flow-rate and column temperature were carefully controlled.

It is well known that the separation power of gas chromatography can be enhanced by careful choice of the column. Moffat *et al.*[20] studied the discriminating power of GC for 62 drugs on eight different columns. Although many stationary phases are available, only a handful seem to be commonly employed in drug analysis.

TABLE I

RETENTION INDICES OF SOME LOCAL ANAESTHETICS ON 5% OV-101, 3% OV-7 AND 3% OV-25 COLUMNS AT TWO DIFFERENT TEMPERATURES

Temperatures (°C) are given in parentheses.

Anaesthetic	*5% OV-101*		*3% OV-7*		*3% OV-25*	
Amylocaine	1582 (150)	1588 (160)	1671 (150)	1676 (160)	1861 (150)	1868 (160)
Prilocaine	1822 (170)	1829 (180)	1969 (170)	1975 (180)	2240 (170)	2248 (180)
Lidocaine	1859 (170)	1866 (180)	1999 (170)	2006 (180)	2266 (170)	2275 (180)
Etidocaine	2024 (180)	2030 (190)	2130 (170)	2138 (180)	2368 (170)	2377 (180)
Trimecaine	1968 (180)	1974 (190)	2108 (180)	2114 (190)	2379 (180)	2388 (190)
Butanilicaine	2008 (180)	2013 (190)	2170 (180)	2178 (190)	2481 (180)	2492 (190)
Procaine	1998 (180)	2005 (190)	2177 (180)	2184 (190)	2528 (180)	2539 (190)
Mepivacaine	2042 (180)	2049 (190)	2202 (180)	2213 (190)	2521 (180)	2538 (190)
Tetracaine	2224 (210)	2229 (220)	2388 (210)	2394 (220)	2713 (210)	2723 (220)
Pramocaine	2263 (210)	2270 (220)	2419 (210)	2427 (220)	2725 (210)	2736 (220)
Oxybuprocaine	2388 (210)	2392 (220)	2566 (210)	2571 (220)	2913 (210)	2922 (220)

TABLE II

INTER-COLUMN RETENTION INDEX DIFFERENCES FOR SOME LOCAL ANAESTHETICS

Temperatures (°C) are given in parentheses.

Anaesthetic	*OV-7 — OV-101*		*OV-25 — OV-7*		*OV-25 — OV-101*	
Amylocaine	89 (150)	88 (160)	190 (150)	192 (160)	279 (150)	280 (160)
Prilocaine	147 (170)	146 (180)	271 (170)	273 (180)	418 (170)	419 (180)
Lidocaine	140 (170)	140 (180)	267 (170)	269 (180)	407 (170)	409 (180)
Etidocaine		114 (180)	238 (170)	239 (180)		353 (180)
Trimecaine	140 (180)	140 (190)	271 (180)	274 (190)	411 (180)	414 (190)
Butanilicaine	162 (180)	165 (190)	311 (180)	314 (190)	473 (180)	479 (190)
Procaine	179 (180)	179 (190)	351 (180)	355 (190)	530 (180)	534 (190)
Mepivacaine	160 (180)	164 (190)	319 (180)	325 (190)	479 (180)	489 (190)
Tetracaine	164 (210)	165 (220)	325 (210)	329 (220)	489 (210)	494 (220)
Pramocaine	156 (210)	157 (220)	306 (210)	309 (220)	462 (210)	466 (220)
Oxybuprocaine	178 (210)	179 (220)	347 (210)	351 (220)	525 (210)	530 (220)

For retention index studies the liquid phases SE-30, OV-1, OV-17 and QF-1 are generally preferred[22,23], while Apiezon L-KOH and PEG-KOH are often used for doping agents[24–26]. Since the last two phases should not be used for the analysis of local anaesthetics containing an ester link, in this study the retention indices of the local anaesthetics were determined using the "popular" phases OV-7 and OV-101 and the more polar OV-25. Except for Amylocaine, most of the local anaesthetics were determined at 180°C, while the retention indices of Tetracaine, Pramocaine and Oxybuprocaine were measured at 210°C. The specificity of the inter-column retention differences is clearly demonstrated in Table II where in most cases only minor differences are noted between the values for each compound at the two temperatures.

Since local anaesthetics containing an ester link and especially Procaine[27] are unstable under strongly alkaline conditions, all extractions were done from biological fluids buffered to pH 9.2. Moreover, since the horse possesses relatively active

TABLE III

EXTRACTION RECOVERIES (%) AND ROUTINE DETECTION LIMITS (ng/ml) FOR SOME LOCAL ANAESTHETICS IN HORSE URINE AND PLASMA

Anaesthetic	*Horse plasma*			*Horse urine*	
	1 Extraction	*2 Extractions*	*Detection limit*	*2 Extractions*	*Detection limit*
Amylocaine	46 ± 11	64 ± 12	10	59 ± 8	15–20
Prilocaine	87 ± 8	96 ± 8	5	94 ± 8	5–10
Lidocaine	86 ± 7	95 ± 6	5	89 ± 4	5–10
Etidocaine	70 ± 8	92 ± 4	2.5	99 ± 5	5–10
Mepivacaine	75 ± 4	91 ± 6	5	85 ± 6	5–10
Pramocaine	64 ± 5	74 ± 4	5	93 ± 7*	10
Trimecaine	78 ± 5	94 ± 4	5–10	93 ± 5	5–10
Tetracaine	78 ± 9	99 ± 4	20	88 ± 5	20
Oxybuprocaine	58 ± 6	77 ± 6	20–25	77 ± 5	25
Butanilicaine	69 ± 7	80 ± 10	40–45	92 ± 5	40–45
Procaine	30 ± 5	49 ± 5	20–25	48 ± 6	20–25

* Human urine.

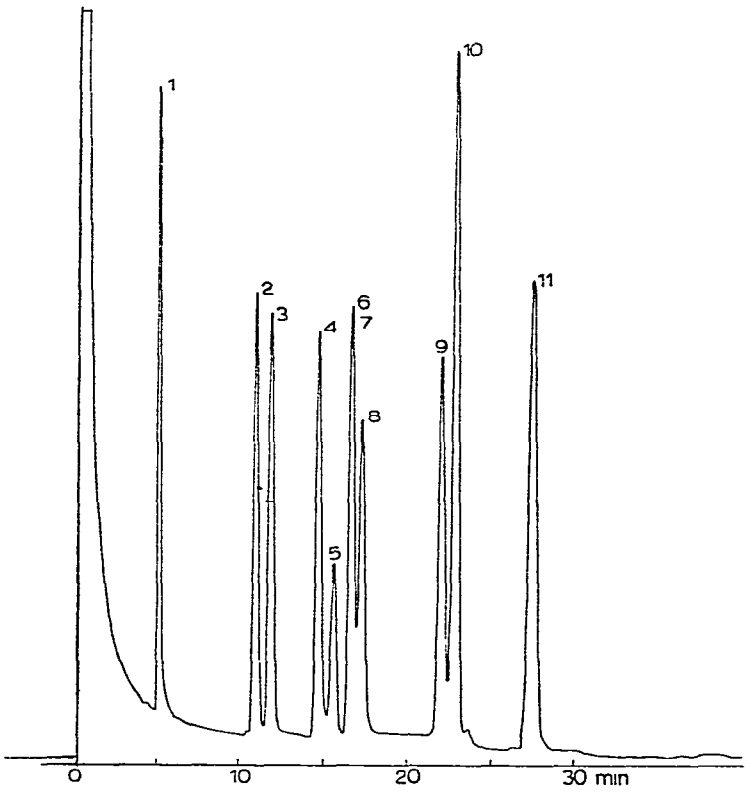

Fig. 2. Gas chromatography of local anaesthetics on column B with temperature programming from 140° to 200°C at 3°/min. Peaks: 1 = Amylocaine; 2 = Prilocaine; 3 = Lidocaine; 4 = Etidocaine; 5 = Trimecaine; 6 = Procaine; 7 = Butanilicaine; 8 = Mepivacaine; 9 = Tetracaine; 10 = Pramocaine; 11 = Oxybuprocaine.

plasma esterases, significant hydrolysis of drugs containing ester links could occur in plasma[28]. Therefore complete and rapid deactivation of these esterases should be done by adding Echothiophate iodide in the blood sampling tubes[4,5].

The mean recoveries and standard deviations (three concentrations, $n = 18$) of eleven local anaesthetics are given in Table III. Several of these compounds are quantitatively recovered after two extractions. Although relatively poor recovery results are obtained for Amylocaine and Procaine, the detection limits with the nitrogen specific detector are in the ppb range using 2 ml of biological fluid.

A GC run for the local anaesthetics studied here using a 2-m column [3% OV-7, Chromosorb W (80–100 mesh)] with temperature programming and nitrogen specific detection is presented in Fig. 2.

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REFERENCES

1 B. E. Pape, R. Whiting, K. M. Parker and R. Mitra, *Clin. Chem.*, 24 (1978) 2020.
2 R. F. Adams, F. L. Vandemark and G. Schmidt, *Clin. Chim. Acta*, 69 (1976) 515.
3 K. K. Adjepon-Yamoah and L. F. Prescott, *J. Pharm. Pharmacol.*, 26 (1974) 889.
4 R. H. Smith, M. A. Brewster, J. A. Macdonald and D. S. Thompson, *Clin. Chem.*, 24 (1978)1599.
5 J. E. O'Brien, V. Abbey, O. Hinsvark, J. Perel and M. Finster, *J. Pharm. Sci.*, 68 (1979) 75.
6 B. J. Kline and M. F. Martin, *J. Pharm. Sci.*, 67 (1978) 887.
7 E. Zylber-Katz, L. Granit and M. Levy, *Clin. Chem.*, 24 (1978) 1573.
8 R. L. Nation, E. J. Triggs and M. Selig, *J. Chromatogr.*, 116 (1976) 188.
9 D. W. Holt, R. J. Flanagan, A. M. Hayler and M. Loizou, *J. Chromatogr.*, 169 (1979) 295.
10 G. Caille, J. Lelorier, Y. Latour and J. G. Besner, *J. Pharm. Sci.*, 66 (1977) 1383.
11 M. T. Rosseel and M. G. Bogaert, *J. Chromatogr.*, 154 (1978) 99.
12 J. D. Cameron, *Clin. Chim. Acta*, 56 (1974) 307.
13 T. Tobin, J. W. Blake, L. Sturma, S. Arnett and J. Truelove, *Amer. J. Vet. Res.*, 38 (1977) 637.
14 J. A. Evans and M. B. T. Lambert, *Vet. Rec.*, 95 (1974) 316.
15 T. Tobin and J. W. Blake, *Brit. J. Sports Med.*, 10 (1976) 109.
16 R. Suzuki, M. Murata, K. Kamei and A. Momose, *Yakugaku Zasshi*, 93 (1973) 942.
17 R. V. Smith, *Int. Lab.*, Nov.–Dec. (1976) 71.
18 E. Kovats, *Helv. Chim. Acta*, 41 (1958) 1915.
19 R. Kaiser, *Chromatographia*, 3 (1970) 134.
20 R. Kaiser, *Chromatographia*, 3 (1970) 388.
21 A. C. Moffat, A. H. Stead and K. W. Smalldon, *J. Chromatogr.*, 90 (1974) 19.
22 L. Kazyak and R. Permisohn, *J. Forensic Sci.*, 15 (1970) 346.
23 H. Berninger and M. R. Möller, *Arch. Toxicol.*, 37 (1977) 295.
24 A. H. Stead, A. C. Moffat, B. Caddy, F. Fish and D. Scott, *J. Chromatogr.*, 84 (1973) 392.
25 M. Donike and D. Stratman, *Z. Anal. Chem.*, 279 (1976) 129.
26 B. Caddy, F. Fish and D. Scott, *Chromatographia*, 6 (1973) 251.
27 T. Tobin, C. Y. Tai and S. Arnett, *Res. Commun. Chem. Pathol., Pharmacol.*, 11 (1975) 187.
28 T. Tobin, J. W. Blake, C. Y. Tai and S. Arnett, *Amer. J. Vet. Res.*, 38 (1977) 637.