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GAS CHROMATOGRAPHIC DETERMINATION OF PROPOXYPHENE AND NORPROPOXYPHENE IN PLASMA

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SUMMARY

Propoxyphene and its major metabolite norpropoxyphene have been determined simultaneously by using gas chromatography. In order to avoid the on-column decomposition of the propoxyphene, derivatives were formed with the aid of lithium aluminium hydride, which cleaves the propionic acid ester of propoxyphene and reduces the norpropoxyphene amide. Promethazine was used as an internal standard.

Propoxyphene and norpropoxyphene levels in plasma were determined in samples from six male volunteers receiving a single oral dose of 150 mg of propoxyphene hydrochloride in a sustained-release form (Abalgin Retard®).

INTRODUCTION

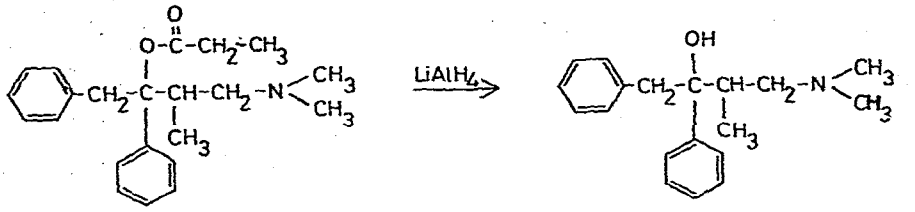
In recent years, several papers have been published on the determination of propoxyphene in plasma and in most instances[1–10] gas chromatography was employed. Only a few of the authors[1–3] mentioned the on-column decomposition of the drug. Sparacino *et al.*[1] reported that the nature of the solid support and of the silanization agent are decisive for this breakdown. However, we have experienced that even columns which do not decompose propoxyphene immediately do so progressively after a few months' routine work, and silanization does not improve the results.

In order to overcome these problems, we decided to prepare a derivative of propoxyphene. The cleavage of the propionic acid ester leads to the formation of a chromatographically stable tertiary alcohol (Fig. 1), and the reagent used, lithium aluminium hydride, also reacts with norpropoxyphene amide, yielding 4-(methylpropylamino)-1,2-diphenyl-3-methyl-2-butanol (Fig. 2). The retention times of the reacted compounds are shorter than those of the unreacted drugs (Table I).

EXPERIMENTAL AND RESULTS

Chemicals and reagents

Propoxyphene and norpropoxyphene. An aqueous stock solution containing 2.5 μg/ml of *d*-propoxyphene hydrochloride and 5 μg/ml of N-desmethylpropoxyphene maleate (norpropoxyphene) is stable for several months when stored at 4°.



d-Propoxyphen

Fig. 1. Reaction of propoxyphene with lithium aluminium hydride.

Internal standard. An aqueous solution of promethazine hydrochloride (Dott. Bonapace et Cie, Milan, Italy) (25 μg/ml) must be prepared freshly every day.

Derivatization agent. Lithium aluminium hydride (Riedel de Haën, Seelze-Hannover, G.F.R.) (100 mg) was shaken with 10 ml of dried diethyl ether for 15 min. When the excess of the hydride has settled, the solution is ready for use.

Other chemicals. Redistilled *n*-butyl chloride, dried distilled diethyl ether, distilled water, carbon tetrachloride (Uvasol, Merck, Darmstadt, G.F.R.), 1 *M* carbonate buffer [pH 9.8 (ref. 11)], 0.2 *N* hydrochloric acid, 4 *N* sodium hydroxide solution and a 2% solution of trimethylamine in acetone were used.

Glassware

Centrifuge tubes of 15-ml volume with screw-caps were silanized as described

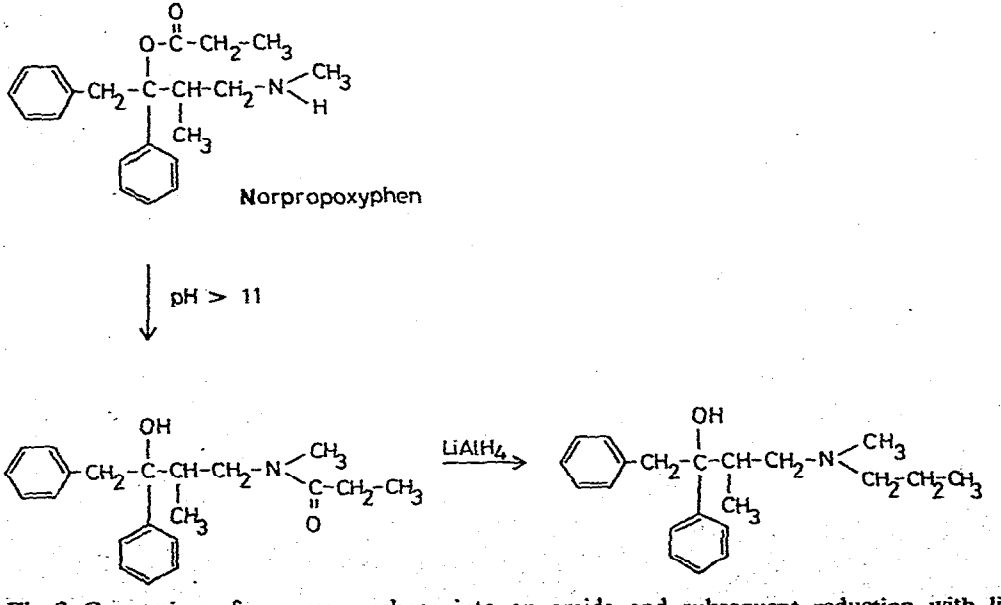


Fig. 2. Conversion of norpropoxyphene into an amide and subsequent reduction with lithium aluminium hydride.

by Walle and Ehrsson[12] and washed before use with the 2% solution of trimethylamine in acetone in order to prevent adhesion of the amines to the glass.

Gas–liquid chromatography (GLC)

The GLC analysis was performed on a Varian Aerograph Model 2100 chromatograph equipped with a flame ionization detector. The U-shaped glass columns (1.8 m × 2 mm I.D.) were silanized. The packing material consisted of 3% OV-17 on Chromosorb W AW DMCS, 80–100 mesh. The temperature of the oven was 180°, injection port 200° and detector 250°. The carrier gas was nitrogen at a flow-rate of 40 ml/min. The hydrogen flow-rate was 40 ml/min and the oxygen flow-rate 200 ml/min. The detector sensitivity was $8–64 \cdot 10^{-12}$ A/mV at full scale.

Sample handling

Blood samples were drawn into heparinized tubes, centrifuged and the plasma was decanted and stored at −24°. This storage does not interfere with the analytical results[4,10].

Extraction procedure

The extraction procedure was a modification of the method described by Verebely and Inturrisi[6]. To 5.00 ml of plasma were added 0.200 ml of the solution of the internal standard, 1.00 ml of carbonate buffer and 5.00 ml of *n*-butyl chloride. The plasma was extracted by vertical rotation (32 rpm) for 15 min and centrifuged immediately for 5 min at 1700 *g*.

After freezing (−24°), the upper phase (*n*-butyl chloride) was decanted into another tube containing 2.00 ml of 0.2 *N* hydrochloric acid. The residue was washed with 1.0 ml of *n*-butyl chloride, and the combined *n*-butyl chloride phases were shaken automatically for 5 min with the acidic phase. After centrifugation for 5 min, the *n*-butyl chloride phase was discarded and the acidic phase washed by shaking it for 5 min with 5.0 ml of diethyl ether. The diethyl ether was discarded after centrifugation and the residue washed with 1.0 ml of *n*-butyl chloride.

TABLE I

RETENTION TIMES OF PROPOXYPHENE, NORPROPOXYPHENE AND PROMETHAZINE

Compound	*Retention time (min)*	
	Untreated	*$LiAlH_4$ treated*
Propoxyphene	12.1	8.0
Norpropoxyphene	82.0	13.0
Promethazine	23.3	20.3

The aqueous phase was made alkaline by the addition of 0.300 ml of 4 *N* sodium hydroxide solution and 15 min later was extracted with 1.00 ml of *n*-butyl chloride by shaking for 5 min. After centrifugation for 5 min, the aqueous phase was frozen and the *n*-butyl chloride decanted. Lithium aluminium hydride in diethyl ether (0.500 ml) was added and the samples were left for 15 min in a water-bath (35–40°).

Excess of lithium aluminium hydride was destroyed by the addition of 0.200 ml of water and, after centrifugation, the water was frozen. The organic phase was decanted and evaporated to dryness in vacuum. The tubes were washed with three drops of *n*-butyl chloride and the *n*-butyl chloride was evaporated. The residue was dissolved in 10 μl of carbon tetrachloride and 1 μl of the solution was injected into the gas chromatograph.

Quantitation

Peak heights were measured and the ratios of propoxyphene and norpropoxyphene to the internal standard calculated. Four standards at two concentrations were made every day in order to construct calibration graphs. All values were calculated as propoxyphene hydrochloride equivalents expressed in nanograms.

Linearity and precision

Linearity of the peak-height ratio was established by the assay of 18 plasma samples containing known amounts of propoxyphene and norpropoxyphene, six samples for each of the three levels used. The results are shown in Table II.

TABLE II

LINEARITY AND PRECISION

Parameter	*Propoxyphene*			*Norpropoxyphene*		
Plasma concentration (ng/ml)	20	55	90	40	110	180
Peak-height ratio (mean for $n = 6$)	0.052	0.168	0.294	0.064	0.183	0.303
Standard deviation of peak-height ratio	0.0063	0.0136	0.0301	0.0059	0.0190	0.0266
(% of mean)	(12)	(8)	(10)	(9)	(10)	(9)
Correlation coefficient ($n = 18$)	0.984			0.984		

It can be seen from the correlation coefficients that the linearity is excellent, while the precision, as expressed by the standard deviation, must be considered satisfactory in view of the very low plasma concentrations.

Later experience showed that the slight positive intercepts on the ordinate by the calibration lines resulting from this experiment (5 ng for propoxyphene and 2 ng for norpropoxyphene) were fortuitous, so in routine work all standard graphs are made to pass through the origin.

Recovery

Internal standard was added to plasma samples ($n = 6$), taken through the whole procedure and propoxyphene alcohol dissolved in *n*-butyl chloride (corresponding to 50 ng/ml of propoxyphene in plasma) was added just before evaporation. The ratio of the peak heights was calculated to be 100%. The internal standard and propoxyphene (50 ng/ml in plasma) were added to other plasma samples ($n = 6$) and taken through the procedure. The peak-height ratio thus obtained was divided by the 100% ratio, and showed a net recovery of 53%.

DISCUSSION

Experimental conditions

pH. Like other workers, we found it essential to maintain a pH of 9.8 during the extraction from plasma. A higher pH will cause the rearrangement of norpropoxyphene to an amide that cannot be re-extracted from *n*-butyl chloride with hydrochloric acid. On the other hand, when carrying out the final part of the extraction procedure, it is important that the pH is increased to above 11 (refs. 6, 13 and 14) and that the samples are allowed to stand for 15 min before extraction in order to ensure a 100% yield of the amide (see Fig. 2).

Freezing of the samples. The samples are frozen in order to reduce the working time required. In the step just before the formation of the derivative, this procedure also ensures the essential low content of water in the *n*-butyl chloride phase (see below).

Washing of the acid phase with diethyl ether. This procedure will remove cholesterol, which cannot be eliminated with *n*-hexane as used by Verebely and Inturrisi[6]. The cholesterol has a retention time of about 2½ h under the GLC conditions mentioned.

Derivatization of propoxyphene

Comparing the GLC retention times and the IR spectra of the hydrochlorides (Fig. 3), we found the derivative of propoxyphene to be identical with *d*-4-(dimethylamino)-1,2-diphenyl-3-methyl-2-butanol, synthesized by Ravensberg GmbH. To investigate whether this alcohol from propoxyphene is formed *in vivo*, we analyzed some samples without the formation of the derivative. Plasma samples were obtained from 10 male volunteers 3 h after administration of 150 mg of timed-release propoxyphene hydrochloride (Abalgin Retard®). The concentration of propoxyphene in the pooled plasma samples was 45 ng/ml, and we were not able to detect the alcohol (the detection limit is *ca.* 2 ng/ml).

Derivatization of norpropoxyphene

Norpropoxyphene has to be converted into the amide before reaction with lithium aluminium hydride, otherwise two reaction products will be formed. Under

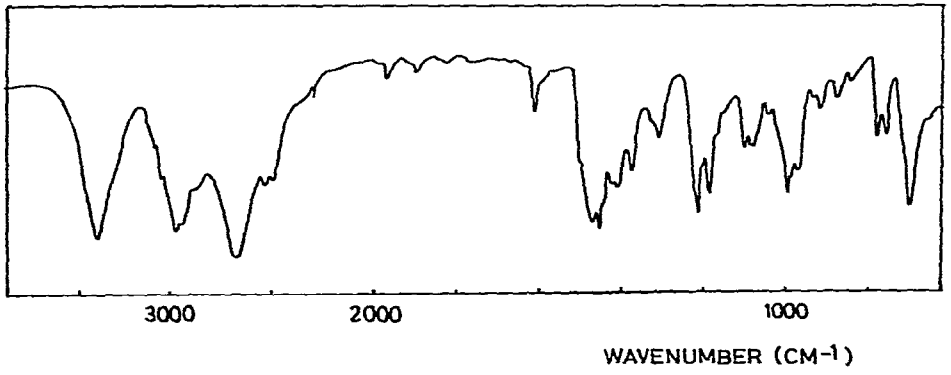


Fig. 3. IR spectrum of the derivative of propoxyphene identical with *d*-4-(dimethylamino)-1,2-diphenyl-3-methyl-2-butanol.

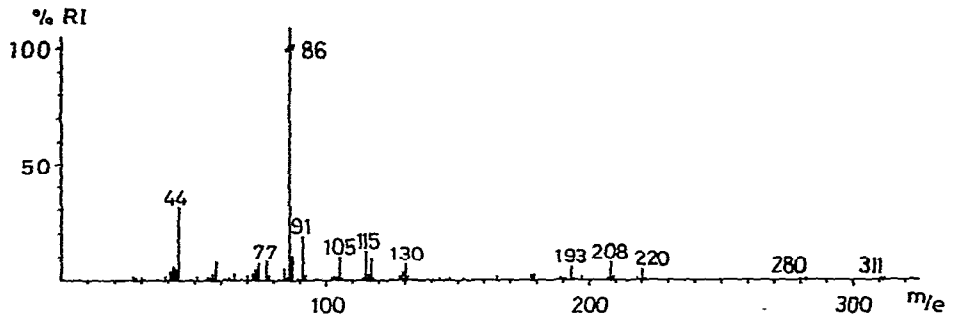


Fig. 4. Mass spectrum of the derivative of norpropoxyphene amide identical with 4-(methylpropylamino)-1,2-diphenyl-3-methyl-2-butanol.

the conditions described (pH above 11 and the samples allowed to stand for 15 min before extraction), only one product is formed. This derivative was identified by mass spectrometry as 4-(methylpropylamino)-1,2-diphenyl-3-methyl-2-butanol (see Fig. 4). When analyzing plasma without the formation of the derivative, no peak is seen in the chromatograms with a retention time corresponding to this compound.

Lithium aluminium hydride

As lithium aluminium hydride reacts violently with water, very low water contents in the *n*-butyl chloride phase and in the diethyl ether are essential. In destroying lithium aluminium hydride with water, highly alkaline reaction products are formed and thus all of the amines remain in the *n*-butyl chloride–diethyl ether phase. Promethazine does not react with lithium aluminium hydride.

The formation of the derivative can be performed at the relevant concentrations with the following standard deviations: propoxyphene 4% ($n = 14$) and norpropoxyphene 6% ($n = 15$).

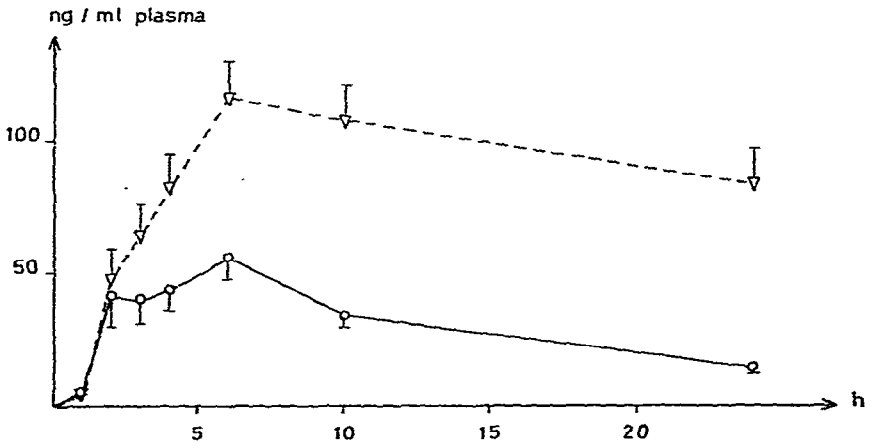


Fig. 5. Plasma levels of propoxyphene (———) and norpropoxyphene (– – –) following an oral dose of 150 mg of propoxyphene hydrochloride in a sustained-release form (Abalgin Retard). The results represent the mean for six male volunteers, and the standard error of the mean is indicated.

Solvent

Carbon tetrachloride is a suitable final solvent as the flame-ionization detector gives a very small response to this compound. Unfortunately, the internal standard is not stable in this solvent. Consequently, the residue has to be dissolved immediately before the injection into the gas chromatograph.

APPLICATION

Six male volunteers received 150 mg of propoxyphene hydrochloride in a sustained-release form (Abalgin Retard). *In vitro*, an amount of 150 mg is released by 26–44%, 56–74% and not less than 80% after 1, 4 and 8 h, respectively[15].

Blood samples were drawn at 0, 1, 2, 3, 4, 6, 10 and 24 h. Apart from an overnight fast, no specific dietary measures were taken except that the consumption of alcohol was not permitted. The results are given in Fig. 5 and examples of the chromatograms are given in Fig. 6.

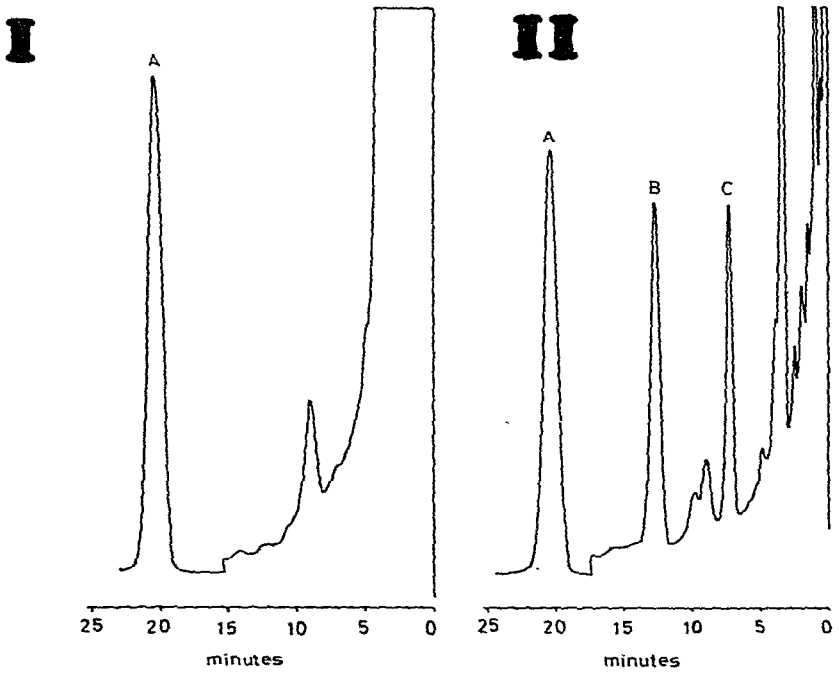


Fig. 6. Gas chromatograms of human plasma extracts. I, Blank containing internal standard (A); II, plasma sample drawn 6 h after administration (A represents the internal standard, B represents 97 ng/ml of norpropoxyphene in plasma and C represents 47 ng/ml of propoxyphene in plasma).

CONCLUSION

Gas chromatography is the method most widely used for the determination of propoxyphene in plasma (and urine), and so on-column decomposition is a problem of general importance. Sullivan *et al.*[8] and Wolen *et al.*[9] avoided the problem by the use of deuterium-labelled internal standards and chemical-ionization mass fragmento-

graphy in combination with gas chromatography. In this instance, the decomposition that may still occur causes no problems as the internal standard will decompose to exactly the same degree.

The method described in this paper avoids the decomposition through derivatization and is thus rendered practicable for the equipment possessed by most analytical laboratories.

The formation of the derivative is not time consuming, the reacted compounds have a shorter retention time than the unreacted drugs, they are chromatographically stable and they do not represent natural metabolites that occur in plasma in detectable amounts. Finally, the standard deviation of the method of about 10% is satisfactory, considering the low plasma concentrations.

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REFERENCES

1 C. M. Sparacino, E. D. Pellizzari, C. E. Cook and M. W. Wall, *J. Chromatogr.*, 77 (1973) 413.
2 J. Manno, N. Jain and R. Forney, *J. Forensic Sci.*, 15 (1970) 403.
3 C. S. Frings and L. B. Foster, *Amer. J. Clin. Pathol.*, 53 (1970) 944.
4 R. L. Wolen and C. M. Gruber, *Anal. Chem.*, 40 (1968) 1243.
5 J. G. Wagner, P. G. Welling, S. B. Roth, E. Sakmar, K. P. Lee and J. E. Walker, *Int. Z. Klin. Pharmakol. Ther. Toxikol.*, 54 (1972) 371.
6 K. Verebely and C. E. Inturrisi, *J. Chromatogr.*, 75 (1973) 195.
7 W. R. Maynard, R. B. Bruce and G. G. Fox, *Anal. Lett.*, 6 (1973) 1005.
8 H. R. Sullivan, J. L. Emmerson, F. J. Marshall, P. G. Wood and R. E. McMahon, *Drug Metab. Dispos.*, 2 (1974) 526.
9 R. L. Wolen, E. A. Ziege and C. M. Gruber, *Clin. Pharmacol. Ther.*, 17 (1975) 15.
10 J. F. Nash, J. F. Bennett, R. J. Bopp, M. K. Brunson and H. R. Sullivan, *J. Pharm. Sci.*, 64 (1975) 429.
11 R. G. Bates and V. E. Bower, in L. Meites (Editor), *Handbook of Analytical Chemistry*, McGraw-Hill, New York, 1st ed., 1963, pp. 11–17.
12 T. Walle and H. Ehrsson, *Acta Pharm. Suecica*, 8 (1971) 27.
13 H. M. Lee, E. G. Scott and A. Pohland, *J. Pharmacol. Exp. Ther.*, 125 (1959) 14.
14 R. E. McMahon, A. S. Ridolfo, H. W. Culp, R. L. Wolen and F. J. Marshall, *Toxicol. Appl. Pharmacol.*, 19 (1971) 427.
15 H. O. Andersen and H. Holmen Christensen, *Dan. Tidsskr. Farm.*, 43 (1969) 117.