Journal of Chromatography, 75 (1973) 195–205

CHROM. 6339

THE SIMULTANEOUS DETERMINATION OF PROPOXYPHENE AND NORPROPOXYPHENE IN HUMAN BIOFLUIDS USING GAS–LIQUID CHROMATOGRAPHY

KARL VEREBELY AND CHARLES E. INTURRISI
Department of Pharmacology, Cornell University Medical College, New York, N.Y. 10021 (U.S.A.)

(Received August 21st, 1972)

SUMMARY

A method employing solvent extraction and gas–liquid chromatography has been developed for the quantitative determination of propoxyphene simultaneously with its major metabolite norpropoxyphene. Norpropoxyphene is analyzed following its conversion to a stable product, norpropoxyphene amide. Using an initial volume of 4 ml the method can detect as little as 0.010 μg of propoxyphene and 0.050 μg of norpropoxyphene per ml of plasma.

Propoxyphene and norpropoxyphene plasma levels were determined in samples from a normal volunteer following a single oral dose of propoxyphene and from patients and volunteers after multiple oral doses. During the first 6 h after drug administration, propoxyphene rapidly disappeared from the plasma of the normal volunteer. In contrast, the plasma level of norpropoxyphene was more persistent. Subjects receiving multiple doses of propoxyphene were found to have a plasma level of norpropoxyphene that was, on the average, three times greater than the corresponding plasma level of propoxyphene.

INTRODUCTION

Propoxyphene is a widely prescribed mild oral analgesic. In man, the major route of biotransformation is mono-N-demethylation of propoxyphene to a secondary amine, norpropoxyphene[1,2] (Fig. 1).

McMAHON *et al.*[2] reported that following the oral administration of ^{14}C-labeled propoxyphene to normal volunteers, the plasma level of propoxyphene declined rapidly during the first few hours while a compound identified as ^{14}C-labeled norpropoxyphene persisted and was still measurable in plasma as late as 100 h after dosage.

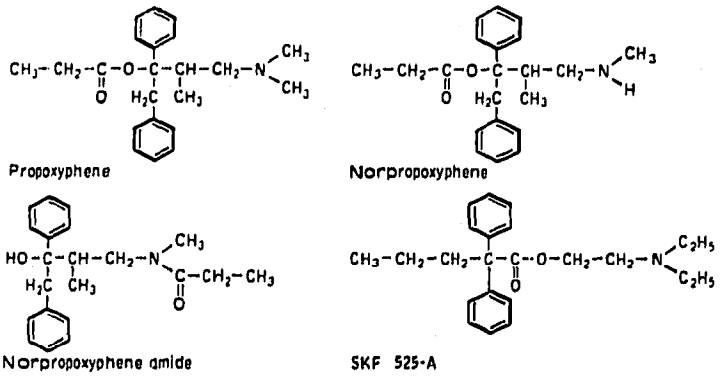


Fig. 1. Structural formulae of propoxyphene, norpropoxyphene, norpropoxyphene amide and SKF 525-A, the internal standard.

In order to investigate more completely the disposition of propoxyphene and its metabolite in man, a specific and sensitive method for the simultaneous determination of propoxyphene and norpropoxyphene in plasma has been developed.

MATERIALS AND METHODS

Chemicals and reagents

d-Propoxyphene hydrochloride and des-N-methyl propoxyphene maleate (norpropoxyphene) were generously supplied by Drs. C. GRUBER, Jr. and J. MCGUIRE of the Lilly Research Laboratories (Indianapolis, Ind.). SKF 525-A (β-diethylaminoethyldiphenylpropylacetate hydrochloride) was a gift of Smith, Kline and French Laboratories (Philadelphia, Pa.).

The solvents used in the extraction procedure were spectral grade chloroform and *n*-hexane, reagent grade *n*-butyl chloride and 99 mol% pure carbon tetrachloride. The *n*-butyl chloride, *n*-hexane and chloroform contain contaminants, that, when concentrated by evaporation, appear as peaks on the chromatogram. Certain of these peaks can interfere with quantitation, particularly of norpropoxyphene. Distillation in a whole glass system was successful in removing these contaminants.

Stock solutions. Aqueous solutions of propoxyphene and norpropoxyphene each at a concentration of 4 μg/ml and SKF 525-A at a concentration of 20 μg/ml are prepared and kept refrigerated.

Sample preparation from plasma. The method described by WOLEN AND GRUBER[3] for the extraction of propoxyphene from plasma was modified to permit the simultaneous recovery of propoxyphene and norpropoxyphene. The method described here requires that norpropoxyphene be determined as its stable conversion product, norpropoxyphene amide.

Into a siliconized centrifuge tube with Teflon®-lined screw cap, add 1 to 4 ml of plasma, 0.05 ml of the solution of the internal standard, SKF 525-A, 0.5 ml of DELORY AND KING's carbonate–bicarbonate buffer[4], 1 *M*, pH 9.8 and one drop of octyl alcohol. The sample is extracted with 10 ml of *n*-butyl chloride by shaking for 5 min in an automatic shaker and centrifuged for 5 min at 1500 r.p.m. The *n*-butyl

chloride phase (upper) is carefully removed to avoid disturbing the interface. It should be emphasized that contamination from the aqueous–organic interface produces an interference peak which emerges from the gas–liquid chromatographic (GLC) column at the zone of norpropoxyphene amide. The *n*-butyl chloride phase is extracted with 5 ml of 0.2 *N* HCl by shaking for 7 min. After centrifugation for 3 min the *n*-butyl chloride (upper) phase is aspirated and discarded. The acid phase is washed by shaking for 3 min with 5 ml of *n*-hexane followed by centrifugation for 3 min. The *n*-hexane (upper) phase is discarded. The washed acid phase is made alkaline by the addition of three drops of 60% NaOH and mixed well on a Vortex mixer for 10 sec (pH should be 12 by indicator paper). This aqueous phase is extracted with 7 ml of chloroform by shaking for 5 min. After centrifugation for 3 min the aqueous (upper) phase is aspirated and discarded. The chloroform is transferred to a 12-ml siliconized conical centrifuge tube and evaporated to dryness in a multiple flash evaporator with the bath at 50° (Evap-O-Mix, Buchler Corp., Fort Lee, N.J.). The sample is collected in the lower tip of the tube and dissolved in 20 μl of carbon tetrachloride and an appropriate volume up to 4 μl is injected into the gas chromatograph.

Sample preparation from urine. Propoxyphene and norpropoxyphene amide may be recovered by the direct extraction of alkaline urine. To a volume of urine (0.5 to 2.0 ml) add 0.25 ml of the aqueous solution of the internal standard, SKF 525-A and 1 drop of 60% NaOH. Mix well for 10 sec and extract with 7 ml of chloroform. Following evaporation, the sample is reconstituted with 40 μl of carbon tetrachloride and 2 μl are injected into the gas chromatograph. Urine samples were also subjected to hydrolysis by Glusulase® using the method of LEVY AND YAMADA[5]. The concentration of urinary base was determined before (free) and after hydrolysis (total). Subtraction of the free base from the total base yields that portion of the base that was conjugated. If interfering materials are present in the urine the multi-step method described for plasma may produce an extract free of additional peaks.

Gas–liquid chromatographic conditions. The GLC analysis is performed on a Varian Aerograph, Model 1740, equipped with a hydrogen flame ionization detector. The column is a 6-ft. long glass spiral with a 2-mm I.D. The packing consisted of 3% SE-30 on Gas-Chrom Q, 80–100 mesh. The temperature of the detector and flash heater is 240°. The carrier gas is helium at a flow-rate of 33 ml/min. Hydrogen flow was between 32 and 40 ml/min and air between 200 and 250 ml/min. Detector sensitivity is 4×10^{-11} A/mV at full scale. The column oven temperature is 216° for both plasma and urine extracts.

Calibration curves and quantitation. Standard calibration curves were established by adding propoxyphene in the range of 0.2 to 2.0 μg and norpropoxyphene in the range of 0.4 to 2.8 μg to fresh control plasma and proceeding as described above for plasma. The norpropoxyphene standard was contaminated by a compound which emerged at the propoxyphene zone, and therefore separate calibration curves were constructed for propoxyphene and norpropoxyphene. Quantitation is performed by drawing the baseline and measuring the peak height from the midpoint of the baseline to the apex of the peak. The ratio of the peak height of propoxyphene or norpropoxyphene amide to that of the internal standard is calculated. A standard curve is constructed by plotting the peak height ratio against the amount (μg added)

of propoxyphene and norpropoxyphene. Each calibration curve was constructed from triplicate determinations of at least four different points. The standard calibration curves for propoxyphene and norpropoxyphene are linear within the range indicated (Fig. 2). The standard calibration curves are also linear when extended to values as low as 0.040 μg for propoxyphene and 0.200 μg for norpropoxyphene (not shown in Fig. 2).

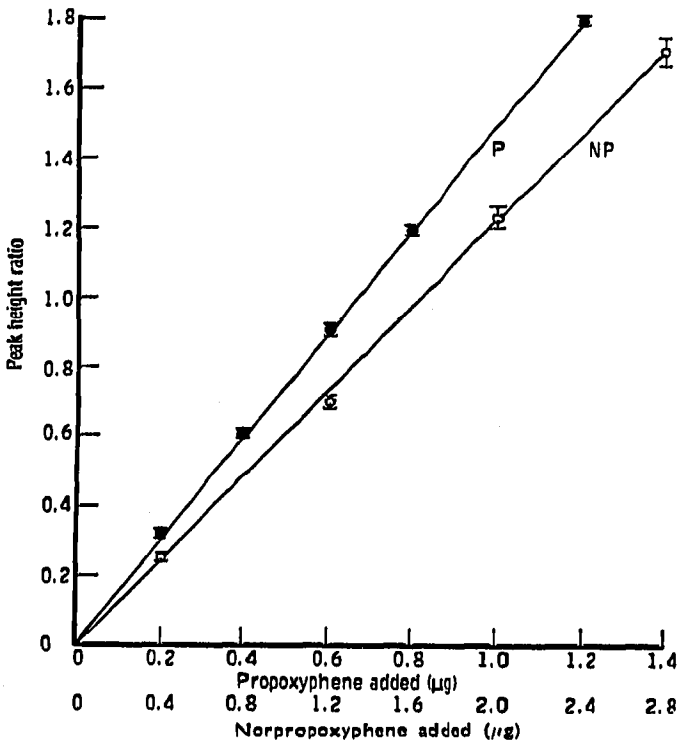


Fig. 2. Standard calibration curves for propoxyphene and norpropoxyphene recovered from plasma. Each point represents the mean of three determinations. I indicate the range of these determinations.

Standard calibration curves were established by the addition to control urine of propoxyphene and norpropoxyphene in the range of 0.4 to 1.2 μg and 2 to 10 μg, respectively, and proceeding as described above for urine.

Sample handling. Blood samples from volunteers and patients were drawn into tubes containing sodium citrate or heparin. The blood was centrifuged and the plasma recovered and analyzed immediately or kept frozen at −15°. It should be emphasized that a decomposition product of plasma (not identified) may interfere with the quantitation of norpropoxyphene. This interfering substance is not seen in fresh plasma or plasma frozen shortly after collection. Urine samples were analyzed immediately after collection or kept frozen at −15°.

RESULTS AND DISCUSSION

The development of a method for the simultaneous determination of propoxyphene and norpropoxyphene required a consideration of the chemical properties of the drug, its metabolite and the internal standard, SKF 525-A. The chemical structures of the compounds of interest are shown in Fig. 1. Propoxyphene and SKF 525-A are tertiary amines and norpropoxyphene is a secondary amine. These amines are weakly basic compounds and might be expected to have similar partition properties in an aqueous-organic system. Norpropoxyphene amide, however, is a nearly neutral molecule of somewhat different partition properties.

LEE *et al.*[1] first reported that norpropoxyphene is unstable in an alkaline solution. Recently MCMAHON *et al.*[2] showed that at pH 11 or above norpropoxyphene undergoes an intramolecular acyl shift. Cyclization occurs by a nucleophilic mechanism between the partially positively charged carbonyl carbon and the free electron pair of the secondary amine nitrogen. The reaction requires the presence of a base to attract a hydrogen from the amino group and make the extra electron pair on the amine nitrogen even more reactive. A schematic representation of the proposed mechanism for the conversion of norpropoxyphene to norpropoxyphene amide is given in Fig. 3. The cyclic intermediate cleaves spontaneously to yield a stable molecule, norpropoxyphene amide. The structural identity of the intramolecular rearrangement product, norpropoxyphene amide, has been verified by infrared and mass spectroscopy[2].

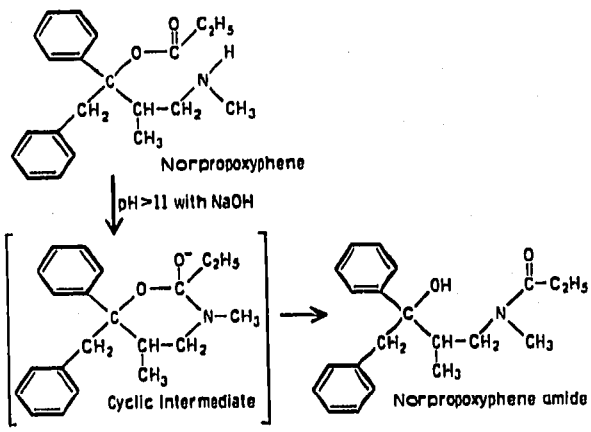
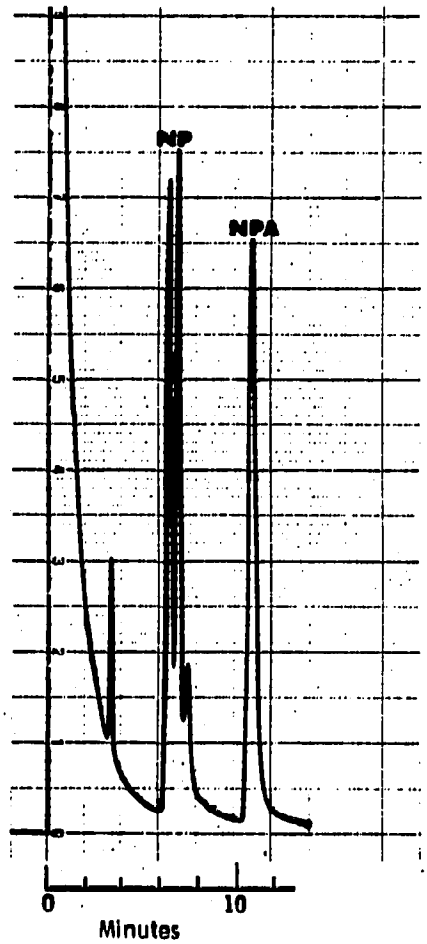


Fig. 3. A proposed mechanism for the conversion of norpropoxyphene to norpropoxyphene amide.

Under the chromatographic conditions described in MATERIALS AND METHODS, norpropoxyphene apparently decomposes into a species producing three peaks (Fig. 4). Thus, norpropoxyphene could not be quantitated using this system. In contrast, norpropoxyphene amide was chromatographically stable producing a single symmetrical chromatographic peak (Fig. 4). Norpropoxyphene amide, however, cannot be recovered through the multi-step plasma extraction procedure. The nearly neutral amide will not readily form a hydrochloride salt and cannot be extracted from *n*-butyl chloride into the acid phase during sample purification. Therefore, conditions were



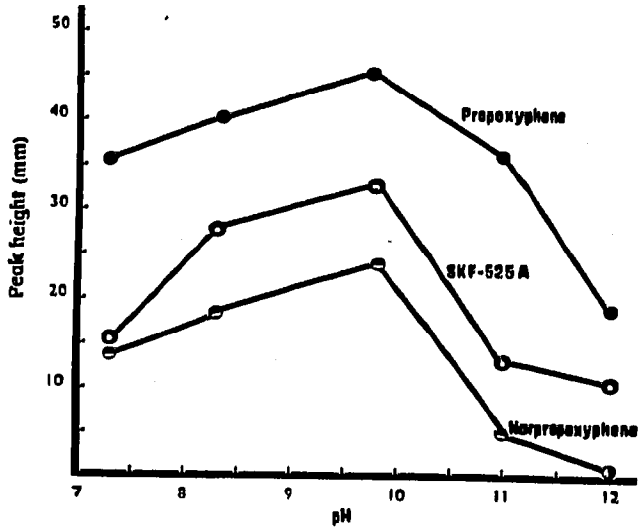


Fig. 4. A chromatogram of norpropoxyphene (NP) and norpropoxyphene amide (NPA). GLC conditions are as described in MATERIALS AND METHODS.

Fig. 5. Determination of the optimal pH for the extraction of propoxyphene, norpropoxyphene and SKF 525-A from plasma. A standard amount of each of the bases was added to plasma. The plasma was buffered to the pH value indicated and extracted as described in MATERIALS AND METHODS. The peak height value is proportional to the amount of base recovered.

determined wherein norpropoxyphene itself was recovered from plasma and then converted to norpropoxyphene amide by the addition of base at the last step in the extraction procedure.

To determine the optimal conditions for the simultaneous extraction of the three bases (propoxyphene, norpropoxyphene and SKF 525-A), a known quantity of each base was added to plasma. The plasma was buffered at selected pH values from 7.3 to 12.0 and the bases were extracted and recovered. The extracts were analyzed by GLC as described above. The amount of each base recovered (expressed as peak height) is plotted as a function of plasma pH (Fig. 5). These results indicate that the recovery of each of the bases from plasma was pH-dependent. The recovery of each of the bases was increased as the pH value of the plasma was raised from 7.3

to 9.8. For example, the recovery of norpropoxyphene was nearly doubled when the plasma pH was raised from 7.3 to 9.8. The optimal pH for the simultaneous extraction of the bases was 9.8. The substantial decrease in the recovery of propoxyphene and SKF 525-A at pH 11 and 12 may be due to increased binding to plasma proteins. The pH-dependent binding to plasma proteins of other weakly basic drugs has been reported by BRODIE *et al.*[6]. The nearly total loss of norpropoxyphene at pH values 11 and 12 resulted from its conversion to norpropoxyphene amide which, as mentioned, cannot be recovered by the plasma extraction procedure.

During the development of this method several organic solvents were tested for their ability to extract the bases from plasma. *n*-Butyl chloride proved to be superior to chloroform, benzene, ethylene chloride or diethyl ether (see also ref. 3). For example, *n*-butyl chloride can nearly completely extract propoxyphene from plasma and at the next step give it up entirely to the acid phase. Chloroform also extracted nearly 100% of the propoxyphene from plasma but retained about 50% after a single extraction with 0.2 *N* hydrochloric acid.

To determine the recovery of the bases, 0.2 μg of propoxyphene, 1.2 μg of norpropoxyphene and 1.0 μg of SKF 525-A were added to 4 ml of control plasma and the recovery carried out as described above. The same quantities, representing an absolute recovery, were extracted from the alkaline aqueous phase (last step in the method). The bases were quantitated by GLC. After correcting for aliquot losses, the recovery from plasma was 97.5% ± 3.1 S.D. for propoxyphene; 69.7% ± 4.1 S.D. for SKF 525-A; and 75.3% ± 4.0 S.D. for norpropoxyphene. These data represent the mean of six determinations. The corrected recovery of propoxyphene from plasma was 97.1% when determined with the use of radioactively labeled [1-^{14}C]-propoxyphene.

At alkaline pH emulsification followed the vigorous shaking of plasma with the organic solvent. The addition of one drop of octyl alcohol prevented emulsification. Octyl alcohol did not interfere with the GLC analysis, since it was not re-extracted into the aqueous phase but was discarded with the *n*-butyl chloride.

The choice of an internal standard was based on previously reported studies[7] in which we found that SKF 525-A was suitable for use as an internal standard in the determination of methadone, a compound structurally related to propoxyphene. As can be seen in Fig. 6, SKF 525-A emerged from the GC column well separated from propoxyphene and norpropoxyphene amide.

Examples of chromatograms obtained under the conditions described in MATERIALS AND METHODS are given in Fig. 6. The multi-step extraction procedure for plasma samples results in an extract that is free of interfering peaks. In most cases it was possible to introduce samples into the gas chromatograph every 15 min.

Figs. 6a and 6b are examples of chromatograms of extracts of plasma to which were added, 1.0 μg of the internal standard (S) and either 0.8 μg of propoxyphene (P) or 2.0 μg of norpropoxyphene. Norpropoxyphene was converted to norpropoxyphene amide (NPA) prior to chromatography. By calculating the ratio of the peak height of propoxyphene or norpropoxyphene amide to that of the internal standard, a single point on each of the standard calibration curves could be determined (see Fig. 2). Fig. 6c shows a chromatogram of an extract of plasma from a subject who received an oral dose of propoxyphene. The internal standard was added directly to the plasma and the extract prepared. The plasma of this subject contained both propoxyphene and

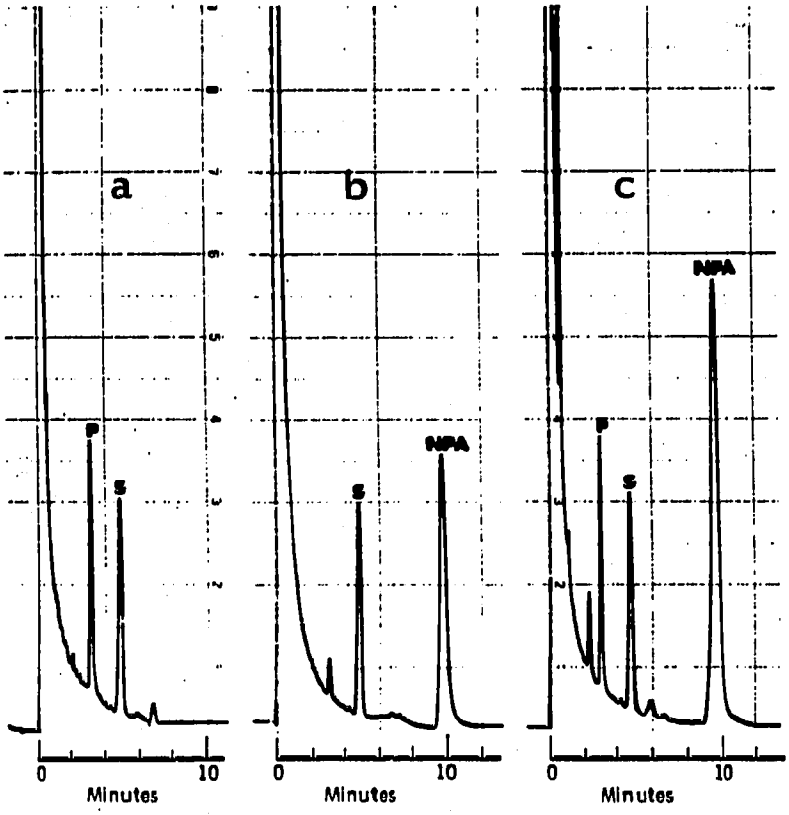


Fig. 6. Chromatograms of human plasma extracts. (a) Extract of control plasma to which 0.8 μg of propoxyphene (P) and 1.0 μg of the internal standard, SKF 525-A (S) were added and the extract prepared. (b) Extract of control plasma to which 2.0 μg of norpropoxyphene and 1.0 μg of the internal standard (S) were added. The norpropoxyphene was converted to norpropoxyphene amide (NPA) prior to chromatography. (c) Extract of plasma from a subject who received an oral dose of propoxyphene. The internal standard was added directly to the plasma and the extract prepared. Abbreviations are as in a and b. Retention times are P = 3.4 min, S = 5.2 min and NPA = 10.1 min. GLC conditions are as described in MATERIALS AND METHODS.

norpropoxyphene determined as norpropoxyphene amide.

Fig. 7 shows the plasma levels of propoxyphene and norpropoxyphene in a normal volunteer who received a single 130-mg oral dose of propoxyphene. 10 ml of blood was collected periodically for the first 6 h and urine samples were collected for the 48 h after drug administration. The plasma level of propoxyphene reached a peak 1 h after drug administration and then rapidly declined in subsequent samples. The peak plasma level of norpropoxyphene occurred at 3 h and the plasma level remained elevated at 6 h. At 6 h after drug administration the plasma level of norpropoxyphene was approximately four times greater than the corresponding plasma level of propoxyphene. In a preliminary report[8] we indicated that the half-life for the elimination of norpropoxyphene from plasma was approximately four times longer than the simultaneously determined half-life for the elimination of propoxyphene from plasma. These results are in agreement with McMAHON *et al.*'s[2]

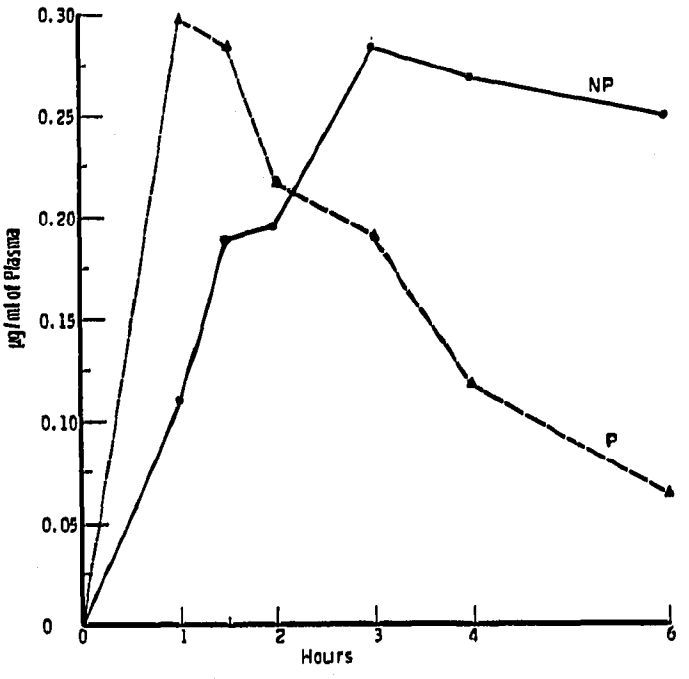


Fig. 7. Plasma levels of propoxyphene (P) and norpropoxyphene (NP) in a normal volunteer following the administration of a single 130-mg oral dose of propoxyphene.

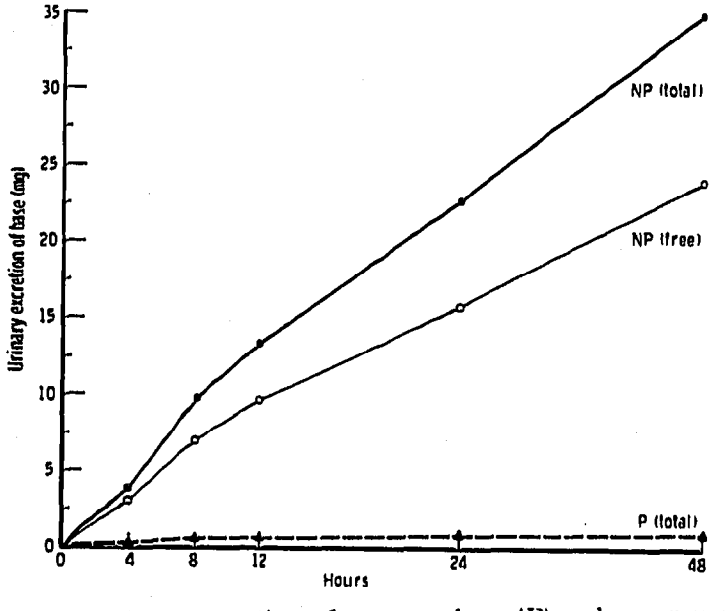


Fig. 8. Urinary excretion of propoxyphene (P) and norpropoxyphene (NP) following a single oral dose of 130 mg of propoxyphene to a normal volunteer. Total NP was determined following Glusulase® hydrolysis of the urine.

report of the very long persistence of ^{14}C-labeled norpropoxyphene in human plasma after a single dose of ^{14}C-labeled propoxyphene.

Fig. 8 represents the cumulative urinary excretion of propoxyphene and norpropoxyphene. The data indicate that very little unchanged propoxyphene was excreted during the 48-h collection period. Norpropoxyphene was present in each of the urine samples as both the free and conjugated base. The major fraction of norpropoxyphene was excreted as the free base. In the cumulative 48 h urine, 24.0 mg was recovered as free norpropoxyphene, 10.9 mg as conjugated norpropoxyphene and 0.9 mg as free propoxyphene. This represents a total recovery of base equal to 27% of the dose. The release of additional norpropoxyphene following Glusulase® treatment of the urine suggests that a portion of the norpropoxyphene in the urine exists as a glucuronide conjugate (see also ref. 2). In urine, no evidence was found of a conjugated form of propoxyphene. The very small quantity of propoxyphene found in the urine indicates that N-demethylation is a prerequisite for the elimination of propoxyphene.

TABLE I

PLASMA LEVELS OF PROPOXYPHENE (P) AND NORPROPOXYPHENE (NP) IN PATIENTS AND VOLUNTEERS RECEIVING MULTIPLE ORAL DOSES OF PROPOXYPHENE

Subject	*mg/dose*	*Number doses per day*	*Total daily dose (mg)*	*Period of administration*	*Plasma concentration (μg/ml)*					
					Hours after administration of propoxyphene					
					2		*4*		*6*	
					P	*NP*	*P*	*NP*	*P*	*NP*
Cancer patient (MB)	65	3	195	2 months	0.746	3.010				
Cancer patient (BB)	65	3	195	2 weeks	0.275	0.750				
Cancer patient (PJ)	130	6	780	1 year	0.866	3.100	0.734	3.230		
Volunteer (MF)	65	3	195	4 days	0.241	0.593	0.204	0.600	0.143	0.575
Volunteer (KV)	130	3	390	4 days	0.849	1.070	0.698	1.168	0.682	1.240

Table I contains the values of the plasma concentrations of propoxyphene and norpropoxyphene determined in subjects and volunteers who had received multiple doses of propoxyphene prior to the dose followed in the survey. When compared to the plasma levels of drug and metabolite seen after a single dose (Fig. 7), the data in Table I suggest that both propoxyphene and norpropoxyphene accumulate in plasma following multiple doses. Clearly norpropoxyphene, consistent with its slower elimination from plasma[2,8], accumulates to a greater extent. On the average the plasma level of norpropoxyphene was three times greater than the corresponding plasma level of propoxyphene. The ratio of the level of norpropoxyphene to propoxyphene in these plasma samples ranged from 1.2 to 4.2 (Table I).

The method we have described permits the simultaneous determination of propoxyphene and its metabolite, norpropoxyphene, in human plasma and urine. This method should be useful in studies of the disposition of propoxyphene in man. In particular, the method will aid in studies of the liver N-demethylating enzyme system in man by allowing the measurement of both the substrate (propoxyphene) and the product (norpropoxyphene) of this enzymatic reaction.

ACKNOWLEDGEMENTS

This study represents a portion of the work carried out by KARL VEREBELY in partial fulfillment of the requirements for the degree of Doctor of Philosophy at Cornell University Graduate School of Medical Sciences. Supported by NIGMS Grant No. GM-00099.

The authors wish to thank Dr. R. HOUDE and A. ROGERS, R.N. for their assistance in the volunteer and patient studies and Dr. A. HAYES for allowing us to use the GC equipment.

REFERENCES

1 H. M. LEE, E. G. SCOTT AND A. POHLAND, *J. Pharmacol. Exp. Ther.*, 125 (1959) 14.
2 R. E. MCMAHON, A. S. RIDOLFO, H. W. CULP, R. L. WOLEN AND F. J. MARSHALL, *Toxicol. Appl. Pharmacol.*, 19 (1971) 427.
3 R. L. WOLEN AND C. M. GRUBER, JR., *Anal. Chem.*, 40 (1968) 1243.
4 G. E. DELORY AND E. J. KING, *Biochem. J.*, 39 (1945) 245.
5 G. LEVY AND H. YAMADA, *J. Pharm. Sci.*, 60 (1971) 215.
6 B. B. BRODIE, S. UDENFRIEND AND J. BAER, *J. Biol. Chem.*, 168 (1947) 299.
7 C. E. INTURRISI AND K. VEREBELY, *J. Chromatogr.*, 65 (1972) 361.
8 K. VEREBELY AND C. E. INTURRISI, *Fed. Proc., Fed. Amer. Soc. Exp. Biol.*, 31 (1972) 538.