J Forensic Sci, Jan. 1972, Vol. 17, No. 1

J. E. Wallace,[1] *Ph.D.*, *S. L. Ladd*,[1] *Ph.D.*, *and Kenneth Blum*,[2] *Ph.D.*

Determination of Propoxyphene in Biological Materials by Ultraviolet Spectrophotometry and Gas Chromatography

Propoxyphene has received widespread utilization as an analgesic agent. Originally thought to be nontoxic, the compound in recent years has been reported as a contributing agent in numerous intoxications of which several have been lethal [*1*]. The compound is a tertiary amino ester, α-d-4-dimethylamino-1-2-diphenyl-3-methyl-2-butanol propionate. Of the four possible stereoisomers only the α-d form has an analgesic effect [*2–4*]. Since propoxyphene absorbs ultraviolet radiation in the manner characteristic of benzenoid compounds, methods which utilize ultraviolet spectra of unconverted propoxyphene have little application to the analysis of this compound in biological systems [*5*].

A thin layer chromatographic procedure that identifies alkaline extractable drugs in urine, including propoxyphene, was developed by Cochin and Daly [*6*]. Wolen and Gruber [*7*] and Manno et al [*8*] used gas chromatography to detect propoxyphene in plasma, and Kazyak and Knoblock [*9*] applied the technique to urine. However, chromatographic techniques often lack the important characteristic of specificity. Specificity can be enhanced by making derivatives of the parent compound that can also be chromatographed.

The methyl orange colorimetric method of Brodie et al [*10*] has been used for the quantitation of propoxyphene in urine, but it has little or no specificity as many other organic nitrogen bases are determined by the technique. A procedure in which the phenyl groups of propoxyphene are converted to a meta-dinitro derivative does not distinguish propoxyphene from other drugs containing unsubstituted phenyl groups [*11*]. In our laboratory none of these methods was effective for specific quantitative determination of tissue concentrations of the drug. In a recent report, Thompson et al [*12*] described a spectrophotometric determination of propoxyphene by exposing the compound to ultraviolet radiation. However, the procedure appears to be applicable only to the analysis of specimen drug concentrations greater than 10 μg/g. In a previous investigation [*13*] an ultraviolet absorbing compound was obtained after hydrolysis of propoxyphene in concentrated hydrochloric acid and subsequent steam distillation. The derivative has a spectrophotometric sensitivity approximately 50 times as great as that of unchanged

Received for publication 7 June 1971; accepted for publication 1 Sept. 1971. The research reported in this paper was conducted by personnel of the USAF School of Aerospace Medicine, Aerospace Medical Division, AFSC, United States Air Force, Brooks Air Force Base, Texas 78235.

[1] Forensic Toxicology Branch, USAF School of Aerospace Medicine, Brooks Air Force Base, Texas 78235.

[2] Department of Experimental Pharmacology, Southwest Foundation for Research and Education, San Antonio, Texas 78228.

propoxyphene. Although the technique has proven useful in determining the drug in biological fluids and tissues, it has the disadvantage of requiring a considerable volume of distillate (50 ml) to ensure quantitative results.

In the method described in this report, steam distillation and the separation of propoxyphene from most other alkaline drugs are not required, and a number of specimens can be analyzed simultaneously. The procedure utilizes a combination of ultraviolet spectrophotometric and gas-liquid chromatographic techniques which provides a specific quantitative method for determining the drug in biological extracts.

Experimental

Instrumentation

Ultraviolet absorption measurements were performed using a Beckman DK-2A ratio recording spectrophotometer with linear presentation of the wavelength. A Beckman IR-9 spectrophotometer was used for infrared absorption measurements. A Barber-Coleman Model 5000 gas chromatograph with a U-shaped 6-ft-long, 4-mm-inside diameter glass column with 2 percent QF-1 on Gas-Chrom Q, 80-100 mesh, was used for gas chromatographic analysis.

Procedure

Volumes of 5 to 10 ml of blood or urine are placed in a 250-ml separatory funnel after being adjusted to a pH of 8.5 to 9.0 with 1*N* sodium hydroxide. Ten-gram amounts of tissue are homogenized with 10 ml of 0.2*M* phosphate buffer, pH 9.0, and transferred to a separatory funnel. A 100 to 150 ml volume of *n*-heptane is added and the mixture is shaken vigorously for 3 min. After separation from the aqueous phase the heptane is filtered through fast-flowing filter paper and the volume recovered is noted and the loss considered in the calculations. Five milliliters of 0.5*N* hydrochloric acid solution is added to the filtered heptane and the mixture shaken vigorously in a separatory funnel for 3 min. Four milliliters of the aqueous layer are transferred to a 250-ml round-bottom flask which has a ground glass neck for attachment to a water cooled reflux condenser. To the flask are also added 8 ml of concentrated hydrochloric acid, after which the flask contents are refluxed for 20 min. After cooling the reaction mixture is neutralized by addition of 16 g of $N_3PO_4 \cdot 12H_2O$ and 2.5 g of sodium bicarbonate. The phosphate salt should be added first and dissolved prior to the addition of the bicarbonate. Then 5 to 10 ml of spectral grade n-heptane is placed in the flask and the contents slowly refluxed for 30 min with constant stirring. After cooling the n-heptane is removed from the flask and its absorbance from 220 to 340 nm is determined against an n-heptane blank by means of a recording spectrophotometer. If analysis at a single wavelength is required, the absorption at 255 nm is determined.

The sensitivity and specificity of the procedure is significantly enhanced by injecting 5 to 10 ml aliquots of the n-heptane solution into a gas chromatograph equipped with a high temperature flame ionization detector and an electrometer capable of detecting signal responses at 1×10^{-12} A. The heptane extract may be concentrated by evaporation prior to injection in order to increase sensitivity if necessary. Optimum conditions for the gas chromatographic identification are outlined in the caption of Fig. 4.

Results

The relationship between the concentration of propoxyphene in blood, urine, or water and the ultraviolet absorbance of the propoxyphene reaction product in n-heptane was found to be linear in the range of 1.0 to 50 μg/ml (Table 1). The ultraviolet absorption

spectra of propoxyphene and its reaction product are shown in Fig. 1. The product has a well defined absorption spectrum which exhibits a characteristic shoulder. Maximum absorption is at 255 nm.

The standard KBr pellet technique was used to compare the infrared spectrum of propoxyphene (Fig. 2) with that of the product (Fig. 3). The spectra show several differences, of which the most important is the absence in the product of absorption bands at 3450 to 3550 cm^{-1}, 1725 to 1730 cm^{-1}, and 1180 to 1190 cm^{-1}. This suggests that the ester group is removed during the reaction sequence. Gas chromatographic analysis of the product resulted in the appearance of two peaks (Fig. 4). Values of the retention ratios of these components relative to the major peak of propoxyphene were 0.39 and 0.42. By

TABLE 1—*Ultraviolet photometric data for propoxyphene reaction product.*

Propoxyphene in Specimen, μg/ml	Absorbance in n-heptane[a]	Absorbance/ Concentration
50	1.855	0.0371
20	0.738	0.0369
15	0.560	0.0373
10	0.365	0.0365
5	0.180	0.0360
2.5	0.094	0.0374
1.0	0.038	0.0380

[a] 255 nm. Reaction product.

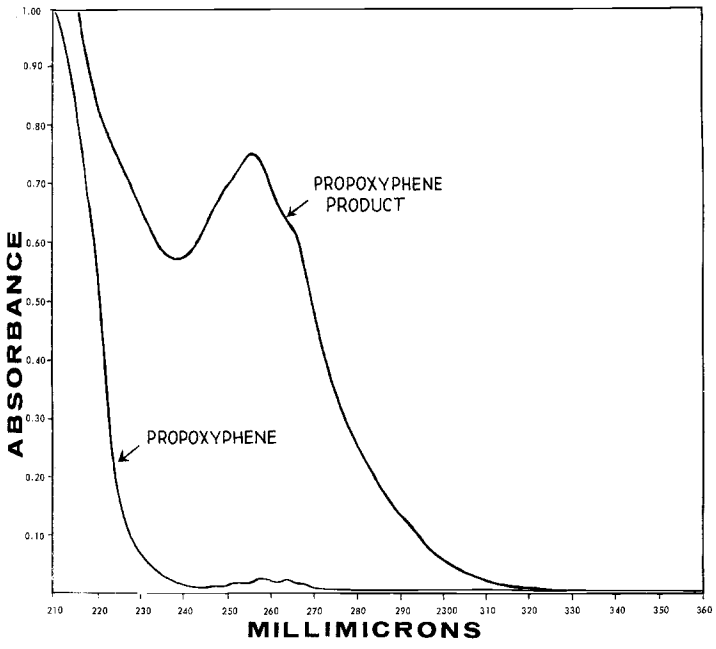

FIG. 1—*Ultraviolet absorption spectra of propoxyphene in dilute hydrochloric acid solution and of the propoxyphene reaction product in n-heptane, each corresponding to a propoxyphene concentration of 20 μg/ml.*

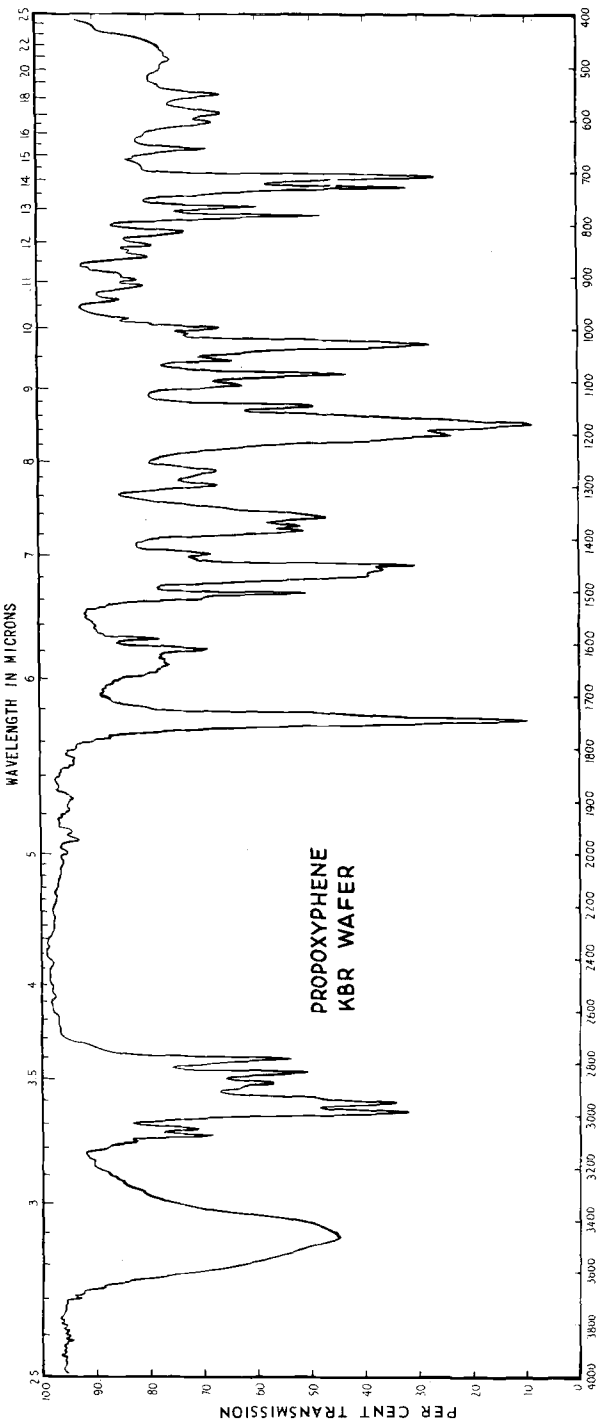

FIG. 2—*Infrared absorption spectrum of propoxyphene hydrochloride, 1 mg in 400 mg potassium bromide.*

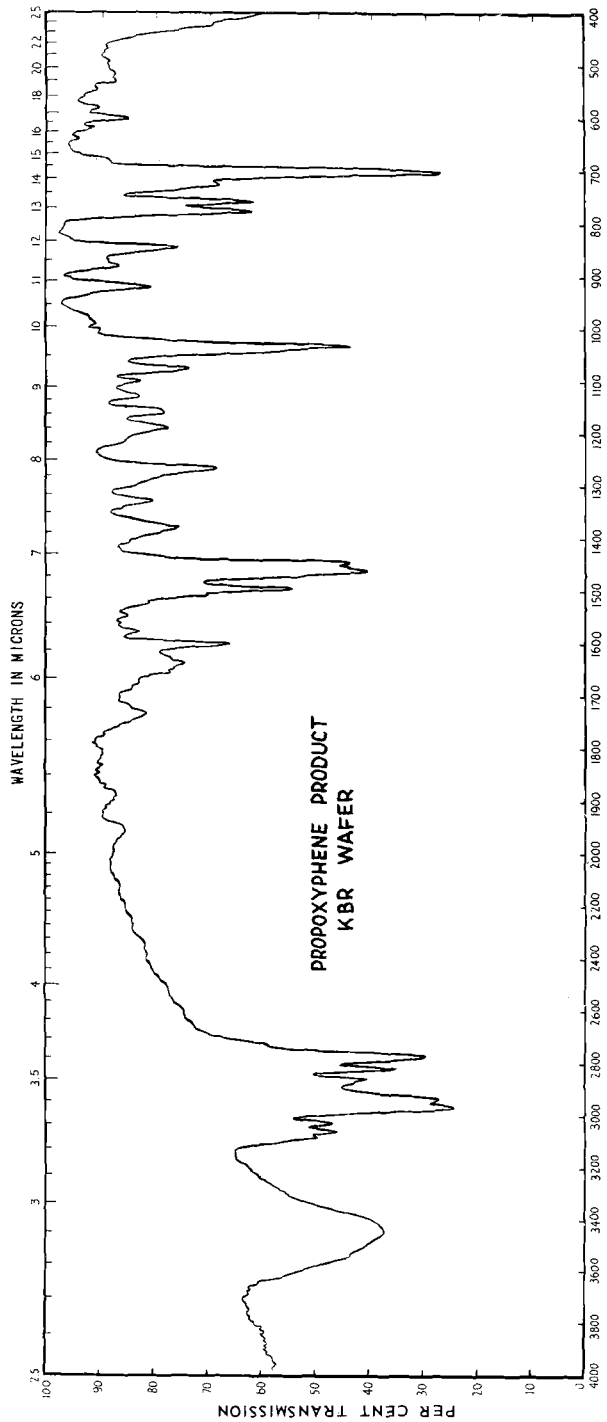

FIG. 3—*Infrared absorption spectrum of the propoxyphene reaction product, 1 mg in 400 mg potassium bromide.*

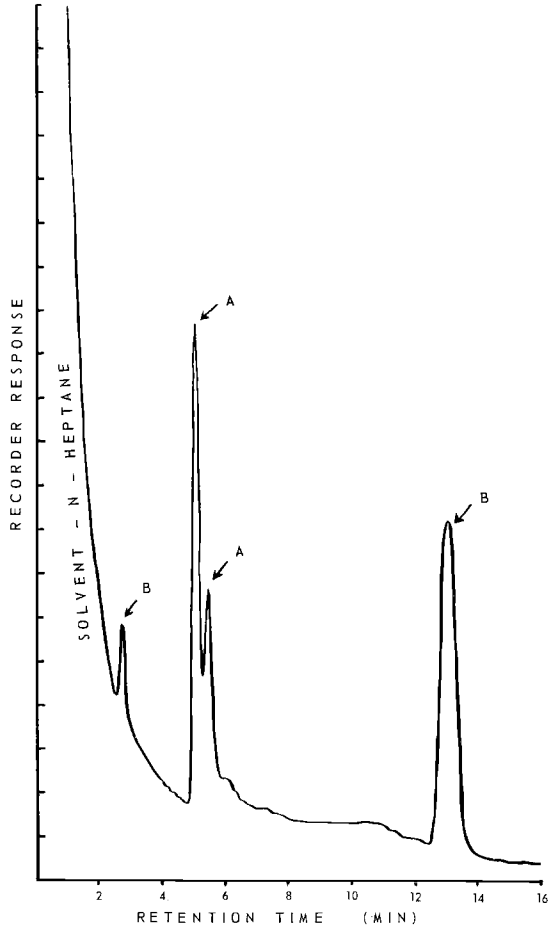

FIG. 4—*Gas chromatogram of propoxyphene (B) and the propoxyphene reaction products (A) on 2% QF-1 on Gas-Chrom Q, 80/100 mesh, in a 6-ft-long U-shaped glass column. Temperatures of column, injector, and detector 220, 280, 285 C, respectively; flow rates of nitrogen, hydrogen, and air 45, 20, and 300 ml/min, respectively. Sensitivity 1 × 10^{-10} A. Concentrations correspond to 1.0 μg of propoxyphene and 0.5 μg of propoxyphene as products.*

using the derivatization procedure, identical retention ratios were observed after propoxyphene had been extracted from the blood, tissue, and urine of individuals who ingested various amounts of the drug (Fig. 5).

Recovery of known amounts of propoxyphene added to biological materials is summarized in Table 2. Standard solutions of the drug were added to 10 ml of whole blood or urine and 10 gm of homogenized liver to provide concentrations from 0.25 to 1.0 mg percent, with the recovery averaging 88 percent.

About 150 commonly used alkaline extractable drugs, including 25 previously reported [*13*], were investigated for interference with the determination of propoxyphene. Several drugs which absorb significantly at 255 nm (phenothiazines) may obscure the absorption

TABLE 2—*Recovery studies of propoxyphene.*[a]

Propoxyphene Added, μg/ml (g)	No. of Determinations	Whole Blood, μg ± S.D.	Urine, μg ± S.D.	Homogenized Liver, μg ± S.D.
10.0	10	9.1 ± 0.5	9.3 ± 0.3	8.9 ± 0.5
5.0	9	4.5 ± 0.3	4.6 ± 0.3	4.4 ± 0.4
2.5	10	2.3 ± 0.2	2.3 ± 0.1	2.2 ± 0.3
avg recovery		91%	92.3%	88.3%

[a] Analysis of 10 ml of urine and blood; 10 g of homogenized liver.

curve of the product in n-heptane, yet none of these compounds has an absorption spectrum which upon careful examination could be mistaken for that of the propoxyphene product.

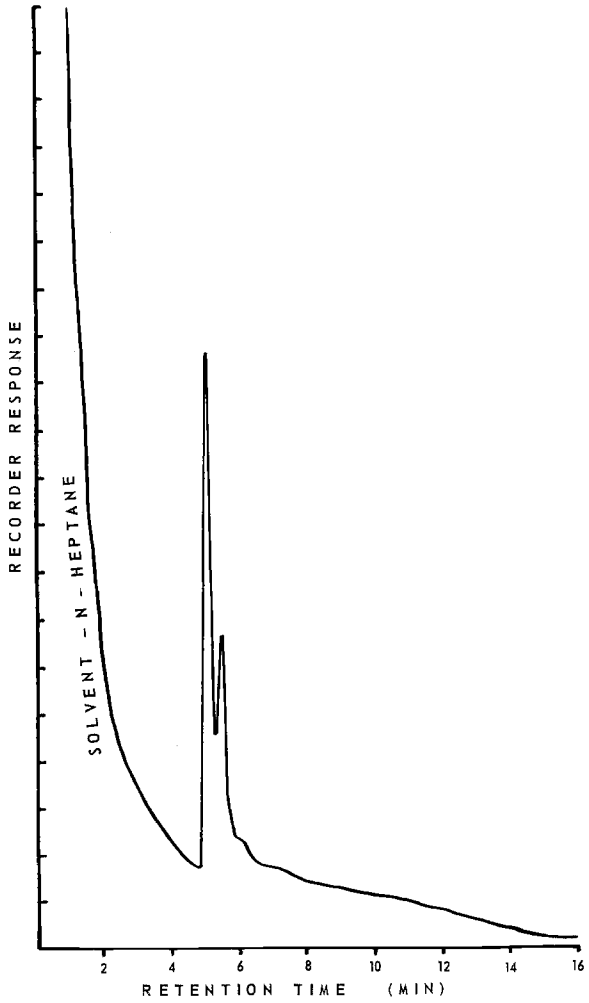

FIG. 5—*Gas chromatogram of the propoxyphene reaction product obtained from propoxyphene extracted from human liver.*

Discussion

During refluxing the pH of the aqueous phase is critical for the quantitative conversion of propoxyphene to the desired product. The tribasic sodium phosphate and sodium bicarbonate neutralize the strong hydrochloric acid solution effectively to a pH of approximately 7.0 ± 0.5, which serves as an optimum range for simultaneous reaction and extraction of the final product into n-heptane. It is advisable to ascertain the pH of the buffered solution prior to refluxing to ensure that it is within the desired range.

The nature of the chemical functional groups associated with the increased capability of the product to absorb ultraviolet radiation was determined from spectrophotometric data. The disappearance of a strong absorption band at 1725 to 1730 cm^{-1} as a result of the reaction indicates that the ester group of propoxyphene is hydrolyzed by acidic catalysis to a tertiary alcohol group. It is feasible that in the presence of concentrated hydrochloric acid the resulting alcohol forms a tertiary chloride. The basis for this hypothesis is the observation that other acids capable of hydrolyzing esters, such as sulfuric, phosphoric, and acetic acid at various concentrations, did not lead to the desired product. The aliphatic chloride at refluxing temperatures in a neutral to slightly alkaline solution is believed to undergo dehydrohalogenation to a product which has an ethylenic group conjugated to one or both phenyl groups. Such a reaction sequence could reasonably explain the increased ultraviolet absorbancy at 255 nm. At this wavelength the molar absorbance would not be significantly increased by the formation of an unconjugated double bond.

A strong band at 700 cm^{-1} combined with absorption at 750 cm^{-1} is considerable evidence for a mono-substituted aromatic ring [*14*,*15*], suggesting that functional groups have not been substituted onto the phenyl groups. The aromatic group is further indicated by the combination of three bands at 1600, 1500, and 1450 cm^{-1} [*16*]. The loss of the strong C—O bond absorption at 1180 cm^{-1} (Fig. 3), which corresponds to that of tertiary alcohols [*17*], supports the dehydrohalogenation mechanism. Since the ultraviolet spectrum of the product is more similar to that of styrene than to that of stilbene [*18*], it seems that of the two possible products in Fig. 6 *A* is most representative of the molecular structure of the propoxyphene product. Upon considering both the spectrophotometric and gas chromatographic data (Figs. 1 and 4), existence of both compounds as products is a possibility.

It was observed that alkaline drugs which have high solubility in hydrocarbon solvents may exist in the final heptane extract. The compounds in this group which absorb ultraviolet radiations strongly in the 255 to 265 nm region interfere in the ultraviolet screeening for the propoxyphene product, as they would in any direct ultraviolet spectrophotometric assay for drugs. With the exception of propoxyphene, however, the reaction sequence did not increase the capability to absorb ultraviolet radiation of any of the many alkaline drugs investigated. Furthermore, a preliminary spectrophotometric scan of the 0.5*N* hydrochloric acid extract prior to acid hydrolysis indicates the presence of substances which could interfere with the analysis of the product at 255 nm. In the investigation of over 150 commonly used alkaline drugs, gas chromatographic analyses differentiated those compounds that interfered spectrophotometrically. No other drug was capable of yielding two peaks of relative retention times identical to those obtained from the propoxyphene product. The pair of chromatographic peaks give a unique order of specificity to this part of the analysis. This combination of instrument utilization provides, therefore, a method with sufficient specificity for clinical as well as forensic purposes.

While it may be assumed, on theoretical grounds, that the spectrophotometric procedure does not distinguish between propoxyphene and its primary metabolic product, des-N-methyl propoxyphene [*19*], that assumption does not vitiate the specificity of the method.

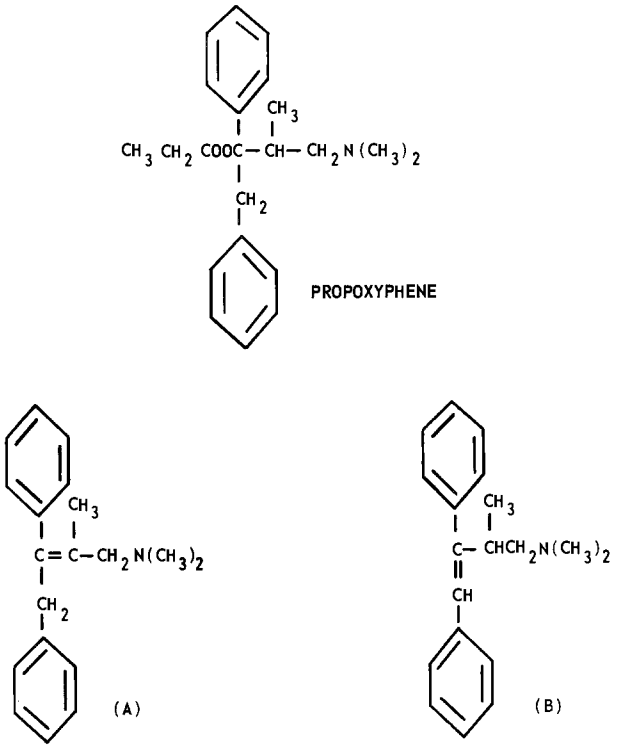

FIG. 6—*Chemical structure of propoxyphene and alternative structures* A *and* B *suggested for the reaction product.*

The gas chromatographic features of propoxyphene metabolites have not been adequately investigated. The procedure has been found to have excellent application in the detection and identification of propoxyphene in biological specimens that also contain large amounts of salicylates and caffeine, substances which are often present in certain analgesic preparations containing propoxyphene.

Summary

A method for quantitative determination of propoxyphene in biological materials is presented. The basis for the procedure is the formation of a derivative which has a high molar absorbancy at 255 nm and adheres to the Beer-Lambert law over a concentration range of 1.0 to 50 μg/ml. When spectrophotometric interference from certain other alkaline drugs is indicated, the derivative can be determined by gas chromatography. With the technique it is possible to measure propoxyphene in the urine of individuals who have taken a single therapeutic oral dose of the drug. The combination of spectrophotometric and gas chromatographic techniques provides a procedure which is sufficiently sensitive and specific for forensic toxicologic purposes.

References

[1] Rehling, C. J., Registry of Human Toxicology, Reports for the 1968, 1969, 1970 Meetings of the American Academy of Forensic Sciences, State Toxicology Laboratory, Auburn, Ala.

[2] Fraser, H. F. and Isbell, H., *Bulletin of Narcotics*, U. N. Dept. of Social Affairs, BNUNA, Vol. 12, 1960, pp. 9–15.

[3] Green, A. F., "Comparative Effects of Analgesics on Pain Threshold Respiratory Frequency and Gastrointestinal Propulsion," *British Journal of Pharmacology*, BJPCB, Vol. 14, 1959, pp. 26–34.
[4] Sadove, M. S., Schiffrin, M. J., and Ali, S. M., "A controlled study of codeine dextropropoxyphene and RO4-1778/1," *American Journal of Medical Science*, AJMCA, Vol. 241, 1961, pp. 103–108.
[5] McBay, A. J. and Algeri, E. J. in *Progress in Chemical Toxicology*, Vol. I, A. Stolman, Ed., Academic Press, New York, 1963, p. 173.
[6] Cochin, J. and Daly, J. W., "Rapid Identification of Analgesic Drugs in Urine with Thin Layer Chromatography," *Experientia*, EXPEA, Vol. 18, 1962, pp. 294–299.
[7] Wolen, R. L. and Gruber, C. M., Jr., "Determination of Propoxyphene in Human Plasma by Gas Chromatography," *Analytical Chemistry*, ANCHA, Vol. 40, 1968, pp. 1243–1246.
[8] Manno, J., Jain, N., and Forney, R., "A Simple Method for the Determination of Propoxyphene in Plasma," *Journal of Forensic Sciences*, JFSCA, Vol. 15, 1970. pp. 158–162.
[9] Kazyak, L. and Knoblock, E. C., "Application of Gas Chromatography to Analytic Toxicology," *Analytical Chemistry*, ANCHA, Vol. 35, 1963, pp. 1448–1452.
[10] Brodie, B. B., Udenfriend, S., and Dill, W. J., "The Estimation of Basic Organic Compounds in Biological Material," *Journal of Biological Chemistry*, JBCHA, Vol. 168, 1947, pp. 335–339.
[11] Personal communication with J. M. McGuire and J. S. Welles, E. Lilly and Company, Indianapolis, Ind., 1964.
[12] Thompson, E., Villaudy, J., Plutchak, B., and Gupta, R. C., "Spectrophotometric Determination of d-Propoxyphene (Darvon) in Liver Tissue," *Journal of Forensic Sciences*, JFSCA, Vol. 15, 1970, pp. 605–609.
[13] Wallace, J. E., Biggs, J. D., and Dahl, E. V., "A Rapid and Specific Spectrophotometric Method for Determining Propoxyphene," *Journal of Forensic Sciences*, JFSCA, Vol. 10, No. 2, 1965, pp. 179–191.
[14] Colthup, N. B., "Spectra-Structure Correlations in the Infra-Red Region," *Journal of Optical Society of America*, JOSAA, Vol. 40, 1950, pp. 397–400.
[15] Randall, H. M., Fowler, R. G., Fuson, N., and Dangl, J. R., *Infrared Determination of Organic Structure*, D. Van Nostrand Co., Inc., New York, 1949.
[16] Bellamy, L. J., *The Infrared Spectra of Complex Molecules*, John Wiley and Sons, Inc., New York, 1958, p. 74.
[17] Bellamy, L. J., *The Infrared Spectra of Complex Molecules*, John Wiley and Sons, Inc., New York, 1958, p. 108.
[18] *The Sadtler Standard Spectra*, published by Sadtler Research Laboratories, Philadelphia, Pa.
[19] Lee, H. M., Scott, E. G., and Pohland, A., "Studies on the Metabolic Degradation of Propoxyphene," *Journal of Pharmacology and Experimental Therapeutics*, JPETA, Vol. 125, 1959, pp. 14–18.

Forensic Toxicology Branch
USAF School of Aerospace Medicine
Brooks Air Force Base, Texas 78235