(11) J. Z. Finklestein, K. Tittle, R. Meshnik, and J. Weiner, *Cancer Chemother. Rep.*, **59**, 571 (1975).
(12) C. Y. Chiou, *J. Pharm. Sci.*, **64**, 469 (1975).
(13) C. Y. Chiou and B. V. R. Sastry, *Biochem. Pharmacol.*, **17**, 805 (1968).
(14) P. P. Rowell and C. Y. Chiou, *ibid.*, **25**, 1093 (1976).
(15) G. R. Ham and T. T. Tuck, in "Methods in Enzymology," vol. 5, S. P. Colowick and N. O. Kaplan, Eds., Academic, New York, N.Y., 1962, pp. 90–119.
(16) J. Z. Finklestein, E. Arima, P. E. Byfield, J. E. Byfield, and E. W. Fonkalsrud, *Cancer Chemother. Rep.*, **57**, 405 (1973).
(17) R. C. Speth and D. E. Schmidt, *Fed. Proc.*, **33**, 477 (1974).
(18) C. Y. Chiou, *Eur. J. Pharmacol.*, **13**, 367 (1971).
(19) C. Y. Chiou and B. V. R. Sastry, *Arch. Int. Pharmacodyn. Ther.*, **181**, 94 (1969).
(20) C. Y. Chiou and B. V. R. Sastry, *J. Pharmacol. Exp. Ther.*, **172**, 351 (1970).
(21) C. Y. Chiou, *Eur. J. Pharmacol.*, **26**, 268 (1974).

ACKNOWLEDGMENTS AND ADDRESSES

Received June 30, 1976, from the *Department of Pharmacology and Therapeutics, College of Medicine, University of Florida, Gainesville, FL 32610.*

Accepted for publication August 11, 1976.

Supported in part by Grant CA-17584, National Cancer Institute, Department of Health, Education, and Welfare, and Grant CH-81, American Cancer Society.

The author is grateful for the dedication and technical assistance of Ms. Marilyn Martin.

Determination of Triprolidine in Human Plasma by Quantitative TLC

R. L. DeANGELIS, M. F. KEARNEY, and R. M. WELCH ˣ

Abstract □ A chromatographic thin-layer fluorescence procedure, with a sensitivity limit of 0.8 ng/ml, is described for the quantitative analysis of triprolidine in human and rat plasma. Following the intravenous administration of 1 mg/kg of triprolidine to rats, the drug distributed rapidly into tissues and was eliminated from plasma with a half-life of 53 min. The method was used to determine the plasma triprolidine levels in 16 normal human volunteers following oral administration of 3.75 mg of triprolidine hydrochloride in 15 ml of a syrup. The drug obtained a mean peak plasma level of 8.2 ng/ml in 2 hr and was eliminated from the plasma with a half-life of 5 hr. Considerable individual variation was observed in the area under the plasma triprolidine level–time curve; values ranged from 19 to 163 ng hr/ml with a mean value of 75 ng hr/ml.

Keyphrases □ Triprolidine—TLC analysis, human and rat plasma, pharmacokinetic profile □ TLC—analysis, triprolidine, human and rat plasma □ Pharmacokinetics—triprolidine, humans and rats □ Antihistamines—triprolidine, TLC analysis, human and rat plasma, pharmacokinetic profile

Triprolidine hydrochloride [(*E*)-2-[3-(1-pyrrolidinyl)-1-*p*-tolylpropenyl]pyridine hydrochloride] is an unusually potent antihistaminic agent used for the treatment of various allergic conditions. Although animal data (1–3) and several clinical reports (4–8) substantiate the efficacy of triprolidine, its disposition is unknown. One report indicated that triprolidine had a duration of action of approximately 4–6 hr (5), but no confirming plasma level data were given.

In another study on the *in vitro* metabolism of ^{14}C-triprolidine by guinea pig liver, the drug was extensively metabolized (9). Other reports described the TLC (10) and GC (11) properties of triprolidine, but no attempt was made to quantitate the drug in tissues. Considering the extensive clinical use of this antihistamine, alone and in combination with other drugs, a sensitive quantitative analysis in biological fluids is needed.

This report describes a sensitive analytical method for quantitating triprolidine in plasma following oral administration of a therapeutic dose to human subjects. The method was used to determine the pharmacokinetic profile of the drug in animals and humans.

EXPERIMENTAL

Materials—The organic solvents utilized to develop the thin-layer plates were reagent grade and were used as received; the solvents employed for the extraction of triprolidine from plasma were glass distilled[1]. A 0.01% stock solution of triprolidine hydrochloride was prepared by dissolving the drug in 1–2 ml of methanol and then adding an appropriate volume of chloroform. From this solution, a working standard of 1 μg/ml was prepared in chloroform. A spray reagent of 2 *M* ammonium bisulfate, prepared every 2 weeks, was employed to induce the fluorescence of the drug on the TLC plate.

Analytical Procedure—Plasma, 1 ml, and 6 ml of dichloroethane were added to a 15-ml conical glass centrifuge tube, mechanically shaken for 15 min, and centrifuged (1000×*g*). Five milliliters of the organic phase was placed in a disposable glass culture tube, the tube was immersed in a water bath at 45°, and the solvent was evaporated to dryness under a nitrogen stream. The residue was redissolved in 0.1 ml of chloroform, and a suitable aliquot (50–80 μl) was spotted on a prescored silica gel plate[2]. Samples were applied to a thin-layer plate with an automatic spotter[3], while the standards (1–5 ng) were spotted by hand using a 10-μl syringe.

Prior to use, each TLC plate was channeled (scored) into 20 units 1 cm wide. The plate was developed to a height of 15 cm in methanol–ammonium hydroxide–chloroform (10:1:89). The solvent system was prepared fresh each day and allowed to equilibrate for 30 min prior to use. After development, the plate was allowed to air dry for 15 min and then sprayed with a 2 *M* aqueous solution of ammonium bisulfate until shiny. After the plate was air dried for about 1 hr, each channel was scanned, and the fluorescent spot representing triprolidine was quantitated.

Instrumentation—Fluorescence measurements were determined by scanning the TLC plate with a spectrodensitometer[4] in the reflectance mode, utilizing only the sample beam that had been passed through a secondary cutoff filter of 405 nm. The fluorophore was excited at 300 nm, and the total emission above 405 nm was read using a density computer[5].

[1] Burdick and Jackson Laboratories.
[2] Siliplate-22, 20 × 20 cm, 0.25 mm, E. M. Laboratories.
[3] Analytical Instrument Specialties multispotter.
[4] Schoeffel SD3000 with reflectance-mode assembly.
[5] Schoeffel SD 30.

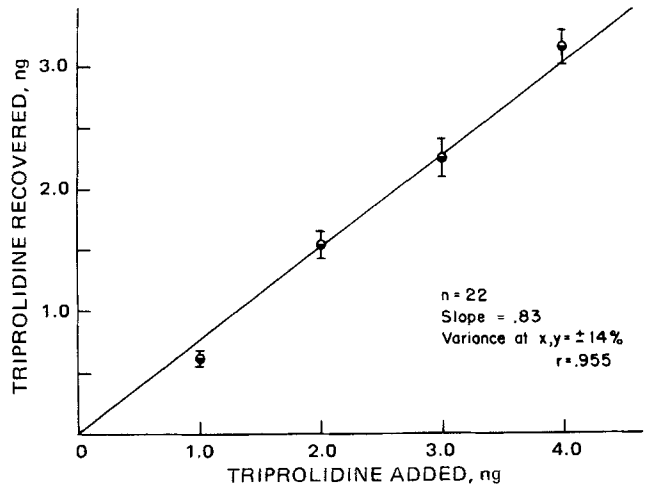

Figure 1—*Recovery of triprolidine from human plasma.*

The area of each peak was simultaneously integrated[6] while recorded on a strip-chart recorder.

Subjects and Protocol—Sixteen male volunteers, with an average age of 23 years and an average weight of 72.5 kg (160 lb), were studied. A complete medical history was taken to eliminate individuals with a history of kidney, liver, or cardiovascular disease. All subjects were fasted 12 hr before and 2 hr after dosing and were free of other medication for 2 weeks prior to triprolidine administration.

Each subject received 15 ml of a syrup[7] containing 3.75 mg of triprolidine hydrochloride followed by 240 ml of water. The syrup was given orally by syringe to ensure complete drug delivery. Seven milliliters of blood was drawn at 0, 0.5, 1, 2, 4, 6, 12, and 24 hr and placed into a Vacutainer containing edetate disodium as the anticoagulant. These samples were centrifuged, and the resulting plasmas were stored in a refrigerator for no more than 24–36 hr before analysis.

RESULTS AND DISCUSSION

Since the pKa of triprolidine is approximately 6.5, it can be extracted quantitatively from plasma into dichloroethane at a physiological pH of 7.4. As shown in Fig. 1, the recovery of 1–4 ng of triprolidine from human plasma was 83% with a variance of 14% at the mean of the linear regression based on 22 determinations. A similar recovery was obtained (n = 29) when higher amounts (5–35 ng) of triprolidine were added to plasma and extracted. The stability of triprolidine was reduced considerably in the presence of a strong base, and the recovery data indicate

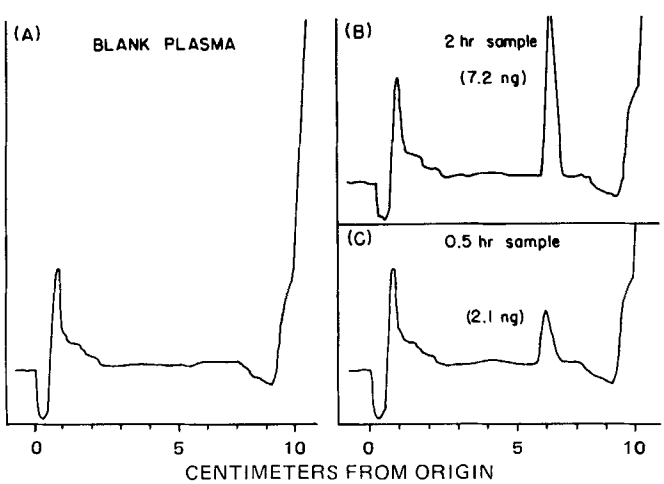

Figure 2—*TLC scan of triprolidine extracted from human plasma. Key: A, control plasma; B, 2.0-hr plasma sample; and C, 0.5-hr plasma sample.*

[6] Autolab System IV.
[7] Actifed Syrup, lot 170-N, Burroughs Wellcome Co.

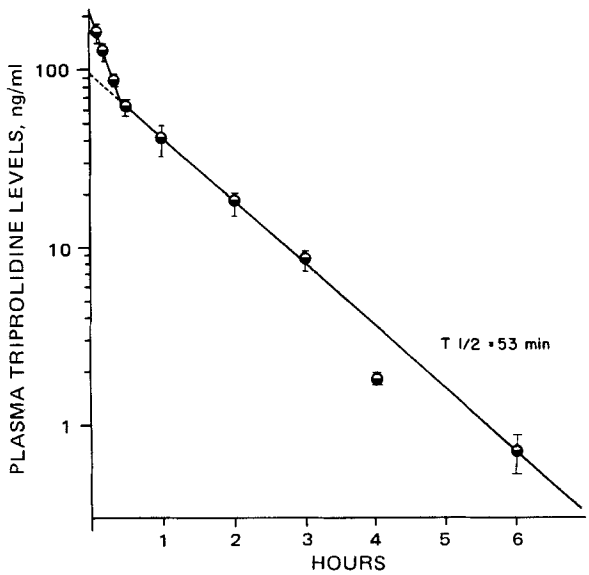

Figure 3—*Disappearance of triprolidine from plasma following intravenous administration of 1 mg of triprolidine hydrochloride/kg to male Sprague–Dawley rats. Results represent the mean ±SE from four rats at each time interval.*

that increasing the pH of plasma above the physiological range is not necessary for good recovery.

Although the exact chemical nature of the fluorophore formed on the plate after spraying triprolidine with ammonium bisulfate is unknown, it is relatively stable to UV irradiation; repeated scans over several hours or direct exposure to irradiation for several minutes did not change the intensity of the fluorescence. However, after 2–3 days of exposure to air, the fluorophore changed its original excitation (300 nm) and emission (>405 nm) wavelengths to 285 and 350 nm, respectively.

A chromatogram of plasma from a human subject prior to and after an oral dose of triprolidine hydrochloride is shown in Fig. 2. Since there was no interfering material in normal human plasma in the region of triprolidine, it was possible to quantitate as little as 0.4 ng of triprolidine spotted on the plate or as little as 0.8 ng of triprolidine/ml of human plasma.

The described procedure was used to determine plasma triprolidine levels in male Sprague–Dawley rats at various times after the administration of 1 mg/kg iv of triprolidine hydrochloride (Fig. 3). The observed plasma concentration (C_p) *versus* time (t) data were fitted to the biexponential equation $C_p = Ae^{-\alpha t} + Be^{-\beta t}$ by least-squares regression analysis, as shown by the solid line. The parameters A and B are zero time intercepts, and α and β are disposition slope constants. The apparent volume of distribution (V_d) of triprolidine was calculated by dividing the

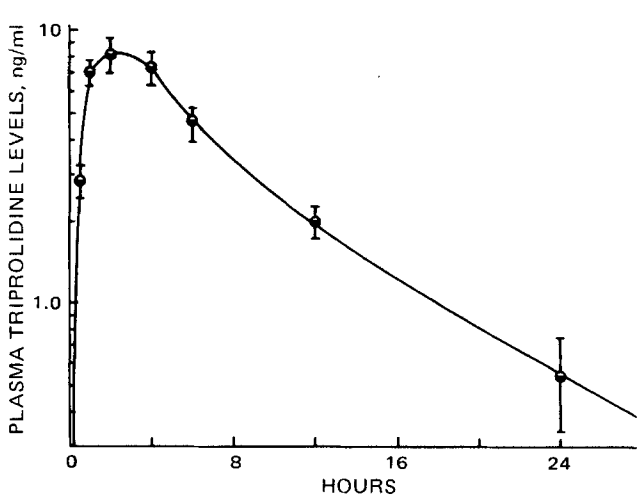

Figure 4—*Plasma triprolidine levels in human subjects following oral administration of a syrup containing 3.75 mg of triprolidine hydrochloride. Results represent the mean ±SE from 16 male volunteers.*

Table I—Plasma Triprolidine Levels in Human Male Subjects following Oral Administration of 15 ml of Syrup Containing 3.75 mg of Triprolidine Hydrochloride

Subject	Age	Weight, kg	Plasma Triprolidine Levels, ng/ml							*AUC*, ng hr/ml
			0.5 hr	1 hr	2 hr	4 hr	6 hr	12 hr	24 hr	
R.J.	27	68	3.3	6.8	6.4	4.2	3.7	3.2	2.0	80
J.H.	26	68	1.6	5.1	4.8	1.8	0.0[a]	0.0[a]	0.0[a]	19
R.F.	20	73	2.0	5.7	8.2	9.7	7.2	2.0	0.0[a]	85
P.D.	18	84	4.5	5.6	4.8	3.8	2.1	0.0[a]	0.0[a]	32
B.J.	18	75	2.9	5.9	6.9	6.5	3.2	0.8	0.0[a]	50
B.S.	20	77	1.3	6.7	11.5	12.5	6.1	1.8	0.0[a]	89
A.O.	21	71	4.2	13.5	14.2	13.3	10.5	5.7	1.7	163
W.K.	25	68	4.0	10.2	16.6	14.1	6.8	1.6	0.0[a]	105
A.L.	23	73	2.1	4.3	5.2	2.8	1.8	0.85	0.0[a]	33
D.G.	29	72	4.4	10.5	17.4	12.7	10.5	7.3	2.2	182
W.S.	21	68	1.3	4.2	3.0	2.3	1.3	0.0[a]	0.0[a]	21
W.L.	29	82	4.8	6.2	6.0	6.5	5.3	2.54	0.0[a]	76
K.W.	21	101	0.0[a]	6.1	8.1	8.8	6.5	4.1	1.6	107
M.D.	21	95	0.0[a]	5.5	5.3	4.9	1.8	0.0[a]	0.0[a]	32
B.S.	20	98	3.5	5.0	3.8	3.5	2.1	0.0[a]	0.0[a]	29
M.G.	20	105	5.2	10.7	9.2	9.5	5.8	2.3	0.0[a]	91

[a] Not detectable; value was considered below the sensitivity of the method and recorded as zero.

intravenous dose by $(AUC)(\beta)$, and the areas under the plasma triprolidine–time curve (AUC) were calculated by applying the trapezoidal rule.

The calculated pharmacokinetic parameters for the rat are: A, 132 ng/ml; α, 0.1 min^{-1}; B, 95 ng/ml; β, 0.0131 min^{-1}; V_d, 9.6 liters/kg; area, 133.5 ng hr/ml; and $T_{1/2}$, 53 min. Following a rapid distribution phase, triprolidine was eliminated from rat plasma with a half-life of 53 min. The large volume of distribution (9.6 liters/kg) indicates the extensive diffusion of this antihistamine into tissues, which is similar to the distribution reported for other antihistamines (12, 13).

Plasma Levels of Triprolidine in Humans—Although triprolidine is used extensively for the treatment of various congestive diseases associated with the release of histamine, plasma drug levels following a therapeutic dose to humans are unknown. Therefore, plasma triprolidine levels were determined in 16 healthy male volunteers following oral administration of 15 ml of a syrup containing 3.75 mg of triprolidine hydrochloride. Individual plasma triprolidine levels at various intervals over 24 hr and the area under the triprolidine plasma–time curve for each subject are shown in Table I. In addition, an average plasma curve for all subjects is shown in Fig. 4.

Plasma triprolidine levels less than 0.8 ng/ml were considered below the sensitivity of the method and were included as zero in calculating the areas under the curves. Of the 16 subjects studied, five individuals had peak plasma levels exceeding 10 ng/ml and only one subject had a peak plasma level below 5 ng/ml. The low plasma levels of this antihistamine in humans are not only related to the low dose administered but are consistent with the data showing a large volume of tissue distribution in rats.

The area under the triprolidine plasma–time curve ranged between 19 and 182 ng hr/ml with a mean value of 75 ng hr/ml. Although triprolidine was detected in plasma within 30 min after its oral administration, the average time required to achieve peak plasma levels was 2 hr. Also, the mean oral plasma half-life of triprolidine was 5.0 hr, but the between-subject variation ranged from 1.5 (Subject J.H.) to 20 (Subject R.J.) hr. Similarly, the mean peak plasma triprolidine level was 8.2 ng/ml but varied from 3 to 17.4 ng/ml. These variations may reflect genetic and environmental factors that influence drug metabolism in humans (14). Also to be considered are individual differences in absorption rates, which are always a problem in an oral study with humans. However, the plasma triprolidine levels obtained following a therapeutic dose of the drug are the lowest reported in humans for any antihistamine.

REFERENCES

(1) P. Armitage, H. Herxheimer, and L. Rosa, *Br. J. Pharmacol.*, **7**, 625 (1952).

(2) A. F. Green, *ibid.*, **8**, 171 (1953).

(3) E. L. Becker, I. Mota, and D. Wong, *ibid.*, **34**, 330 (1968).

(4) R. P. Warm, *Br. Med. J.*, **1**, 1066 (1954).

(5) F. Glassow, *ibid.*, **1**, 217 (1970).

(6) I. G. Popescu, V. Stroescu, and O. Banescu, *Rev. Roum. Med. Int.*, **6**, 291 (1969).

(7) H. P. Wassermann, *Dermatologica*, **130**, 201 (1965).

(8) A. S. E. Fowle, D. T. D. Hughes, and G. J. Knight, *Eur. J. Clin. Pharmacol.*, **3**, 215 (1971).

(9) R. Kuntzman, E. Sernatinger, I. Tsai, and A. Klutch, in "Importance of Fundamental Principles in Drug Evaluation," D. Tedeschi and R. Tedeschi, Eds., Raven, New York, N.Y., 1968, p. 87.

(10) W. W. Fike and I. Sunshine, *Anal. Chem.*, **37**, 127 (1965).

(11) C. R. Fontan, W. C. Smith, and P. L. Kirk, *ibid.*, **35**, 591 (1963).

(12) A. J. Glasko, D. A. McGinty, W. A. Dill, M. L. Wilson, and C. S. Ward, *J. Biol. Chem.*, **179**, 409 (1949).

(13) R. Kuntzman, A. Klutch, I. Tsai, and J. J. Burns, *J. Pharmacol. Exp. Ther.*, **149**, 29 (1965).

(14) J. J. Burns, in "Fundamentals of Drug Metabolism and Disposition," B. LaDu, H. Mandel, and E. Way, Eds., Williams & Wilkins, Baltimore, Md., 1971, p. 340.

ACKNOWLEDGMENTS AND ADDRESSES

Received May 19, 1976, from the *Department of Medicinal Biochemistry, The Wellcome Research Laboratories, Burroughs Wellcome Co., Durham, NC 27709.*

Accepted for publication August 13, 1976.

The authors thank Miss Claudia Taylor for her assistance in the preparation of this manuscript.

[x] To whom inquiries should be directed.