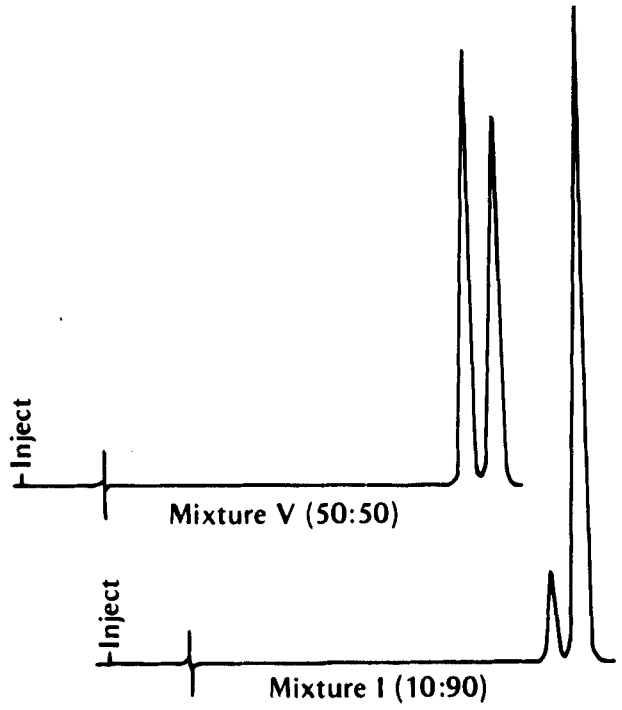

Figure 2—*HPLC chromatograms of mixtures of* (Z)- *and* (E)-*doxepin hydrochloride standards (Table I).*

The precision of the HPLC method was tested by injecting five aliquots of a 15% (*Z*)- and 85% (*E*)-doxepin hydrochloride isomer standard solution. The relative standard deviation was 0.3% for each isomer. The method was calibrated using separate solutions of individual isomers. Excellent linearity of the HPLC response–concentration relationship was demonstrated.

Assay accuracy was investigated by analyzing the five different (*Z*)- and (*E*)-doxepin hydrochloride mixtures of known composition (Fig. 2). The (*Z*)- and (*E*)-isomer content was calculated *via* Eqs. 1 and 2, respectively. The accuracy of the HPLC method is shown by the data presented in Table I. Assay selectivity was challenged by analysis of process-related substances. The results summarized in Table II illustrate the selectivity of the procedure. Long-term reproducibility and adaptability for routine analysis were verified by analyzing doxepin hydrochloride on columns of widely differing assay usage. (For instance, a new column was tested *versus* a 2-year-old column employed in assay work other than doxepin hydrochloride analysis and subjected to methanol deactivation). Equivalent assay data and comparable doxepin isomer resolution were observed in all cases.

The modified HPLC assay for the (*Z*)- and (*E*)-doxepin hydrochloride isomers has been established to be precise, accurate, and selective. The method is amenable to routine laboratory usage, and interlaboratory adoption of the methodology should present no procedural difficulties.

Table II—Retention Times of Process-Related Substances

Compound	Relative Retention Time
6,11-Dihydrodibenz[*b,e*]oxepin-11-one	0.18
Phthalide	0.20
Phenol	0.43
11-(3-Dimethylamino-1-propyl)-6*H*-dibenz[*b,e*]oxepin-11-ol	0.81
(*Z*)-Doxepin	1.00
(*E*)-Doxepin	1.06
2-(Phenoxymethyl)benzoic acid	>1.60

REFERENCES

(1) R. M. Pinder, R. N. Brogden, T. M. Speight, and G. S. Avery, *Drugs*, **13**, 161 (1977).

(2) *Pharmacopeial Forum*, Sept.–Oct. 1978, p. 514.

(3) *Ibid.*, July–Aug. 1978, p. 434.

Sensitive Assay for Determination of Hydroxyzine in Plasma and Its Human Pharmacokinetics

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Abstract □ An assay suitable for hydroxyzine determination in human plasma following therapeutic doses was developed. The method involves GLC and chemical-ionization mass spectrometry of the acetate derivatives of hydroxyzine and of a pentadeuterated analog internal standard. Following administration of 100-mg single oral doses to normal male volunteers, peak plasma concentrations of ~80 ng/ml were observed; the half-life of drug removal was ~3 hr.

Keyphrases □ Hydroxyzine—analysis, GLC–mass spectrometry, human plasma, following therapeutic doses □ Tranquilizers (minor)—hydroxyzine, GLC–mass spectrometric analysis, human plasma, following therapeutic doses □ GLC–mass spectrometry—analysis, hydroxyzine, human plasma

Hydroxyzine, 2-[2-[4-[(4-chlorophenyl)phenylmethyl]-1-piperazinyl]ethoxy]ethanol (I), is an antihistamine compound with antianxiety and antiemetic properties used in the treatment of nervous and emotional disturbances (1), for controlling nausea and vomiting (2), and for preoperative and postoperative sedation and analgesia (3). Procedures for hydroxyzine detection by TLC (4) and electrophoresis (5) do not have the sensitivity necessary for analysis of biological samples. GLC methods have been described (6–8) but the flame-ionization detection was inadequate for plasma samples; use of mass spectrometric detection was suggested (7, 8).

Preliminary studies in this laboratory indicated that the direct analysis of hydroxyzine was complicated by peak tailing with common stationary phases. The oxidation of hydroxyzine to a benzophenone, a product with improved GLC properties, has been examined (9, 10). However, several hydroxyzine metabolites can be expected to form the same benzophenone, rendering such a method nonspecific. Hydroxyzine is metabolized in the rat to *p*-chloro-*p'*-hydroxybenzophenone, *p*-chlorobenzhydrol, [2-(1-piperazinyl)ethoxy]ethanol (norchlorcyclizine), *p*-

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Table I—Analysis of Human Plasma Samples Containing Known Amounts of Hydroxyzine [a]

Amount Added, ng/ml	Amount Found, ng/ml	*RSD*, %
2	2.7	29.6
5	5.0	10.0
25	24.0	7.9
50	49.0	3.7
75	77.3	6.6
100	98.9	5.6

[a] Five samples were examined at each concentration.

chlorobenzophenone, and piperazine (11). Glucuronides of the first two also were identified. The formation of hydroxyzine-*N*-oxide by the rat was reported (12). Metabolism studies have not been conducted in humans.

This report describes a specific and sensitive assay for hydroxyzine determination in human plasma; pharmacokinetic properties were assessed following the administration of a single oral 100-mg dose.

EXPERIMENTAL

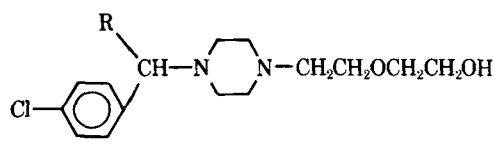
Materials—All solvents were glass distilled[1], and all reagents were ACS grade and used as received. The centrifuge and culture tubes were silylated with 10% dichlorodimethylsilane[2] in toluene. The tubes were rinsed in succession with toluene, methanol, and acetone and were dried at room temperature. Standard hydroxyzine hydrochloride[3] solutions were prepared weekly in methanol at concentrations of 1 mg/ml (free base) and were kept at 4°.

Pentadeuterated Hydroxyzine (II) Synthesis—*p*-Chlorobenzhydryl-d_5 diethyldiamine (1.45 g, 5 mmoles) (13), chloroethoxyethanol (0.62 g, 5 mmoles), and triethylamine (0.5 ml, 6 mmoles) were stirred with toluene (25 ml) and heated at reflux for 48 hr. The reaction mixture was allowed to cool to room temperature. Water (20 ml) was added, and stirring was continued for 20 min. The mixture was filtered, and the aqueous layer was adjusted to pH 8. After phase separation, the aqueous layer was extracted with three 25-ml portions of toluene. The combined toluene phases were washed with two 5-ml portions of dilute hydrochloric acid (pH 6.8) and then reextracted with three 25-ml portions of dilute hydrochloric acid (pH 1).

The aqueous layers were treated with 40% aqueous NaOH to adjust the pH to 11–12 and were extracted four times with 25-ml portions of benzene. The combined benzene extracts were filtered through anhydrous sodium sulfate and then evaporated, leaving the crude product, parts of which were purified by preparative TLC (methanol–water, 67:33). The material having the R_f of hydroxyzine was dissolved in 5 ml of ethyl acetate. Several drops of dry hydrogen chloride gas in isopropanol (6.8%) were added to form the hydrochloride salt, which was separated by centrifugation and washed three times with 10 ml of hexane.

The final product was left under vacuum overnight to remove excess solvent. The identity and the isotopic purity of the hydroxyzine-d_5 thus obtained were established by high-resolution mass spectrometry. The parent ion (M^+) was within 2 millimass units of the theoretical value. The relative abundance of the ion at M − 5 (d_0) was <0.1% of the parent ion, indicating an isotopic purity of >99.9%. The hydroxyzine-d_5 was chemically pure by GLC and by TLC (chloroform–methanol, 9:1).

Assay—Plasma samples (2 ml) in 15-ml centrifuge tubes were mixed with 200 ng of hydroxyzine-d_5 and with 0.25 ml of aqueous 10 *N* NaOH.



I: R = C_6H_5
II: R = C_6D_5

[1] Burdick & Jackson, Muskegon, Mich.
[2] Aldrich, Milwaukee, Wis.
[3] Vistaril, Pfizer, New York, N.Y.

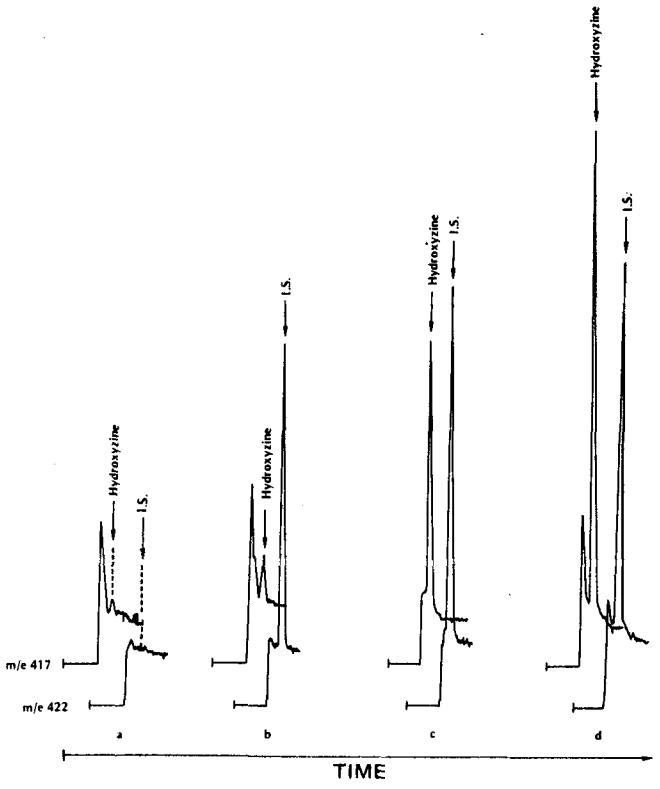


Figure 1—*Selected ion current profiles of control plasma* (a); *control plasma fortified at 2 ng of hydroxyzine/ml* (b), *control plasma fortified at 50 ng of hydroxyzine/ml* (c), *and Subject 1, 2 hr post dose* (d).

The mixture was extracted with hexane (3 × 10 ml), and the hexane extracts were discarded. The aqueous phase was diluted with water (3 ml) and extracted with ether (8 ml). After centrifugation, each tube was dipped in a dry ice–acetone bath to freeze the aqueous phase, and the organic phase was transferred to a 10-ml culture tube and evaporated to dryness by a dry nitrogen stream.

The residues were treated with acetyl chloride (0.1 ml) and vortexed[4] for 30 sec, and the excess acetyl chloride was removed with a dry nitrogen stream. Immediately before analysis, the residues were dissolved in 50 μl of methanol, and aliquots (3–5 μl) were injected into the chromatograph.

Instrumentation—Analyses were carried out on a mass spectrometer equipped for chemical ionization[5]. Samples were introduced by GLC on a 0.8-m × 2-mm i.d. glass column packed with 3% OV-17 on Chromosorb W (80–100 mesh). The column, the injection port, and the transfer lines were kept at 290°. Methane was used as a carrier gas. The ion source temperature was maintained at 165° with a temperature controller[6]. The reagent gas, a mixture of methane and ammonia, was made up by bleeding ammonia directly into the ion source to give a pressure of 0.35–0.40 torr; methane flow through the column was adjusted to give a final pressure of 0.95–1.0 torr in the ion source.

Voltage switching to monitor the ion currents at *m/e* 417 and 422 was controlled by PROMIM units. The signals from the mass spectrometer were recorded on a four-pen recorder[7]. Parameters were adjusted to give maximum sample intensities while unit resolution was maintained.

Calculations—Plasma hydroxyzine concentrations were calculated from the peak height at *m/e* 417 relative to the internal standard peak at *m/e* 422 with reference to a standard curve constructed from plasma samples fortified with known amounts of hydroxyzine.

Human Studies—Hydroxyzine hydrochloride was administered to four healthy male volunteers in the form of commercial 100-mg tablets following an overnight fast. Food was withheld for an additional 2 hr. Blood samples were drawn into heparinized tubes at 0, 0.5, 1, 2, 4, 6, 8, and 24 hr. Plasma was obtained by centrifugation and frozen until assay.

[4] Vortex Genie, Scientific Industries.
[5] Finnigan model 3200.
[6] LFE Corp., Waltham, Mass.
[7] Model HR-4, Houston Instruments, Austin, Tex.

Table II—Human Plasma Concentrations following Oral Administration of 100 mg of Hydroxyzine

Subject[a]	Weight, kg	Nanograms per Milliliter						
		0.5 hr	1 hr	2 hr	4 hr	6 hr	8 hr	24 hr
1	91	<2	<2	74	65	57	38	<2
2	84	<2	23	39	89	65	34	<2
3	77	<2	4	65	82	58	31	<2
4	82	<2	51	86	75	58	38	<2
Mean		<2	20	66	78	59	35	<2

[a] All subjects were male, ages 24–26 years.

Table III—Pharmacokinetic Parameters

Subject	A	α	Absorption Half-Life, hr	B	β	Elimination Half-Life, hr	Lag Time, hr	t_{max}	C_{max}
1	439	1.043	0.66	192	0.203	3.42	1.0	2.9	85
2	467	0.443	1.56	418	0.258	2.68	0.6	3.5	70
3	526	0.670	1.03	362	0.281	2.46	1.0	3.2	85
4	270	0.844	0.82	206	0.208	3.33	0.4	2.6	90
Mean			1.02			2.97	0.7	3.0	82

RESULTS AND DISCUSSION

Hydroxyzine could not be analyzed directly by GLC–mass spectrometry because of poor GLC characteristics at low concentrations due to the polar hydroxy side chain. Attempts to prepare the hexafluoroacetate or the heptafluorobutyrate ester were not successful because hydroxyzine was unstable under the reaction conditions. Acetate ester formation using acetyl chloride proved to be simple and rapid, requiring only a few seconds at room temperature. The excess derivatizing agent was conveniently removed by evaporation. Although hydroxyzine acetate was less volatile than hydroxyzine, it exhibited excellent GLC properties, giving sharp and symmetric peaks at low concentrations (Fig. 1).

Initial studies showed that the use of ammonia as a reagent gas for chemical ionization favored the formation of the hydroxyzine acetate adduct ion (M + H). This has been the case also for the structurally related meclizine (13) and for some amines (14). Chemical ionization and detection of the pseudomolecular ion obviated difficulties in specificity arising from the known animal (11) hydroxyzine metabolites.

Calibration curves for hydroxyzine in plasma were linear from 2 to 100 ng/ml. One factor limiting the assay sensitivity was the presence of a small peak at *m/e* 417 and at the hydroxyzine acetate retention time in plasma of untreated human volunteers. With 2 ml of plasma, this peak was equivalent to 0.59 ± 0.01 ng of hydroxyzine/ml (Fig. 1). The relative standard deviation of the assay was high at the lowest detectable hydroxyzine level but was 10% or less at concentrations of ≥5 ng/ml (Table I). Hydroxyzine recovery from human plasma samples containing 50 ng/ml was 82%.

Plasma concentrations in four subjects receiving single oral 100-mg hydroxyzine doses are given in Table II. The drug appears to be consistently absorbed, giving peak concentrations of ~80 ng/ml 2–4 hr following administration. The data from each subject were applied to an iterative computer program by which the data were fitted to a one-compartment open model represented by:

$$C_t = -Ae^{-\alpha t} + Be^{-\beta t} \qquad \text{(Eq. 1)}$$

Excellent fits were obtained; the kinetic parameters are indicated in Table III. The absorption and elimination half-lives were obtained directly from the rate constants, α and β. Lag time, t_0, was estimated (15) from:

$$t_0 = \frac{\ln A - \ln B}{\alpha - \beta} \qquad \text{(Eq. 2)}$$

This lag time presumably corresponds to the time required for formulation disintegration and drug dissolution and for transport to the absorption site. The time of maximum plasma concentration, t_{max}, was calculated from:

$$t_{max} = \frac{2.303}{\alpha - \beta} \log \frac{\alpha}{\beta} + t_0 \qquad \text{(Eq. 3)}$$

and was applied to Eq. 1 to calculate the maximum plasma concentration.

The mean absorption half-life of 1.02 hr indicates that hydroxyzine is absorbed by humans at a rate typical of many drugs. The mean elimination half-life, 3 hr, is similar to that of the chemically related drug meclizine (13).

REFERENCES

(1) H. L. Goldbert and R. J. Finnerty, *Psychosomatics*, **14**, 38 (1973).

(2) J. C. Snow, *Anesth. Analg.*, **44**, 487 (1965).

(3) A. Matsuki and E. K. Zsigmund, *Agressologie*, **14**, 67 (1973).

(4) S. J. Mule, *J. Chromatogr.*, **55**, 255 (1971).

(5) S. N. Tewari, *Z. Anal. Chem.*, **275**, 31 (1975).

(6) C. Cardini, V. Quercia, and A. Calo, *J. Chromatogr.*, **37**, 190 (1968).

(7) H. Brandenberger, *Pharm. Acta Helv.*, **45**, 394 (1970).

(8) E. van der Kleijn, G. C. Beelen, and M. A. Frederick, *Clin. Chim. Acta*, **34**, 345 (1971).

(9) B. Caddy, F. Fish, and J. Tranter, *Analyst*, **99**, 555 (1974).

(10) P. Hartvig and W. Handl, *Acta Pharm. Suec.*, **12**, 349 (1975).

(11) S. F. Pong and C. L. Huang, *J. Pharm. Sci.*, **63**, 1527 (1974).

(12) J. A. Close, J. G. Gobert, and L. A. M. Rodriguez, *Proc. Eur. Soc. Study Drug Toxic.*, **9**, 144 (1968).

(13) H. G. Fouda, F. C. Falkner, D. C. Hobbs, and E. W. Luther, *Biomed. Mass Spectrom.*, **5**, 491 (1978).

(14) I. Dzidic, *J. Am. Chem. Soc.*, **94**, 8333 (1972).

(15) J. G. Wagner, "Fundamentals of Clinical Pharmacokinetics," Drug Intelligence Publications, Hamilton, Ill., 1975, p. 81.

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