Table I—Minimum Inhibitory Concentration [a] by Agar Dilution Method in Sabouraud's Dextrose Agar

Test Organism	V [b]	VI [c]	Clotrimazole
Candida albicans (two strains)	>256	>512	1
Candida tropicalis	>256	>512	1
Rhodotorula sp.	>256	>512	8
Aspergillus niger	>256	>512	8
Trichophyton mentagrophytes	>256	>512	0.5

[a] Micrograms per milliliter of medium. [b] Target concentration was 256 μg/ml. [c] Target concentration was 512 μg/ml.

mole) of 1.76 *M* phenyllithium in benzene–ether[2]. After stirring at room temperature for 15 min, the reaction was gently refluxed with stirring for 1 hr. The cooled mixture was treated carefully dropwise with 10 ml of saturated aqueous ammonium chloride followed by 20 ml of water. The organic layer was separated, washed with water, dried with anhydrous magnesium sulfate, and evaporated.

The residual oil was then triturated with petroleum ether and filtered, and the solid was crystallized from an ethanol–hexane mixture to provide 4.3 g (73.4%) of white crystals, mp 131–134.5°; UV: λ_{max} 236 (A_m = 13,100), 281 (3000), and 290 (4400) nm; IR (KBr): 3540, 3450, 3065, 1595, 1570, 1445, 1115, and 755 cm^{-1}.

Anal.—Calc. for $C_{19}H_{13}ClO_2$: C, 73.91; H, 4.24; Cl, 11.48. Found: C, 74.45; H, 4.27; Cl, 11.54.

1-Chloro-9-(imidazo-1-yl)-9-phenylxanthene (V) and 1-Chloro-9-(imidazo-2-yl)-9-phenylxanthene (VI)—A mixture of 0.9 g (0.003 mole) of IV and 3.0 g (0.44 mole) of imidazole was heated under a nitrogen atmosphere at 180° for 5 hr. After cooling, the reaction mixture was digested with 30 ml of water, and the insoluble solid was separated by filtration. The solid was dissolved in chloroform, chromatographed on 75 g of a 2-cm silica gel (40–140 mesh) column[3], eluted with ether–chloroform (1:1), and collected in 100-ml fractions. Compound V eluted first followed by VI. Following evaporation of the appropriate fractions, both V and VI were crystallized from ethanol–water.

For V, the yield was 0.5 g (46%), mp 235–237°; R_f 0.7 [silica gel GF, ether–chloroform (1:1 v/v)]; UV: λ_{max} 285 (A_m = 3280) nm; IR (KBr): 3065, 1590, 1570, 1440, 1368 (CN), 1110, 1100, and 755 cm^{-1}; NMR ($CDCl_3$–dimethyl sulfoxide-d_6): δ 6.8–7.4 (m, 13H, aromatic) and 7.5 and 7.6 (overlapping triplet, 1H each, imidazole ring H).

Anal.—Calc. for $C_{22}H_{15}ClN_2O$: C, 73.64; H, 4.21; Cl, 9.88; N, 7.81. Found: C, 73.89; H, 4.31; Cl, 10.00; N, 7.76.

[2] Aldrich Chemical Co.
[3] Baker.

For VI, the yield was 0.1 g (9%), mp 223–225°; R_f 0.3 (same system as for V); UV: λ_{max} 285 (A_m = 3140) nm; IR (KBr): 3070, 2860 (imidazole NH), 1590, 1570, 1440, 1100, 1090, and 755 cm^{-1}; NMR ($CDCl_3$–dimethyl sulfoxide-d_6): δ 6.48 (distorted doublet, 1H, imidazole CH), 6.8–7.4 (m, 13H, aromatic H), and 7.55 (distorted doublet, 1H, imidazole CH); NMR (dimethyl sulfoxide-d_6–D_2O): δ 6.33 (d, imidazole CH), 6.8–7.4 (m, aromatic H), and 7.54 (d, imidazole CH).

Anal.—Calc. for $C_{22}H_{15}ClN_2O$: C, 73.64; H, 4.21; Cl, 9.88; N, 7.81. Found: C, 73.61; H, 4.40; Cl, 9.91; N, 7.66.

Mycology—The antifungal activity of V and VI, using clotrimazole as a reference, was determined by using a Steer's replicator (9) to inoculate plates of Sabouraud's dextrose agar containing twofold serial dilutions of the compounds. The replicator wells contained fungal suspensions at concentrations greater than 6×10^5 colony-forming units/ml. The minimum inhibitory concentration was determined as the lowest concentration that prevented substantial growth of the test organisms. The data summarized in Table I show that neither V nor VI was active against fungi at the concentrations tested.

REFERENCES

(1) K. H. Buchel, E. Regel, and M. Plempel, U.S. pat. 3,660,577 (1972).
(2) P. R. Sawyer, R. N. Brogden, R. M. Pinder, T. M. Speight, and G. S. Avery, *Drugs*, **9**, 424 (1975).
(3) S. Casadio, A. Donetti, and G. Coppi, *J. Pharm. Sci.*, **62**, 773 (1973).
(4) W. Draber, H. Timmler, K. H. Buchel, and M. Plempel, U.S. pat. 3,814,802 (1974).
(5) *Ibid.*, U.S. pat. 3,778,447 (1973).
(6) W. Draber, K. H. Buchel, and M. Plempel, U.S. pat. 3,737,548 (1973).
(7) *Ibid.*, U.S. pat. 3,647,816 (1972).
(8) A. A. Goldberg and A. H. Wragge, *J. Chem. Soc.*, **1958**, 4234.
(9) E. Steers, E. L. Foltz, and B. S. Graves, *Antibiot. Chemother.*, **9**, 307 (1959).

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High-Performance Liquid Chromatographic Determination of (*Z*)- and (*E*)-Doxepin Hydrochloride Isomers

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Received February 15, 1979, from the *Special Testing and Analytical Development Laboratories, Quality Control Division, Pfizer Inc., Groton, CT 06340.* Accepted for publication May 8, 1979.

Abstract □ A high-performance liquid chromatographic method for the determination of (*Z*)- and (*E*)-doxepin hydrochloride isomers was developed. The analysis employs a column packed with spherical silica microparticles (5–6 μm), and a mobile phase of acetonitrile–chloroform–diethylamine (750:250:0.2) permits baseline resolution and simultaneous determination of the (*Z*)- and (*E*)-doxepin isomers. Process-related substances do not interfere. The method is accurate and precise (the relative standard deviation was 0.3% for both isomers). The simple procedure is highly suitable for routine doxepin hydrochloride analysis.

Keyphrases □ Doxepin hydrochloride—isomers, high-performance liquid chromatographic analysis □ Antidepressants—doxepin hydrochloride, isomers, high-performance liquid chromatographic analysis □ High-performance liquid chromatography—analysis, doxepin hydrochloride isomers

Doxepin hydrochloride, 3-dibenz[*b,e*]oxepin-11(6*H*)-ylidene-*N,N*-dimethyl-1-propanamine hydrochloride, is a dual-action antianxiety and antidepression psychotherapeutic agent (1). Chemically, it is a mixture of (*Z*)- and (*E*)-isomers (I*a* and I*b*, respectively) in an ~15:85% ratio. Recently, a GLC procedure (2), which requires an extraction step to convert the hydrochloride to free base, was recommended for doxepin hydrochloride analysis.

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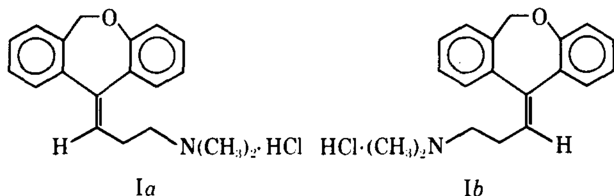
Table I—Recovery of (Z)- and (E)-Doxepin Hydrochloride from Known Mixtures

Mixture	Doxepin Isomer Concentration, mg/ml (Z)-Isomer	(E)-Isomer	Total Isomer Concentration, mg/ml	(Z)-Isomer, % Theory	Determined	(E)-Isomer, % Theory	Determined
I	0.0077	0.0638	0.0715	10.8	10.8	88.3	88.8
II	0.0149	0.0568	0.0717	20.8	20.7	78.5	79.0
III	0.0185	0.0532	0.0717	25.8	25.9	74.1	74.6
IV	0.0221	0.0496	0.0717	30.8	30.7	69.0	69.3
V	0.0367	0.0355	0.0722	50.8	50.7	49.3	49.6



Earlier, a high-performance liquid chromatographic (HPLC) procedure[1] was considered (3) but suffered from insufficient resolution of the two isomers, resulting in poor reproducibility. In the current work, modification of the HPLC column system produced a rapid, simple procedure that consistently afforded reproducible baseline resolution and quantification of the isomers.

EXPERIMENTAL

Chemicals and Reagents—(Z)-Doxepin hydrochloride [specified as 100% (Z)] and (E)-doxepin hydrochloride [specified as 99.4% (E) and 0.6% (Z)] were obtained as crystalline substances[2]. The following solvents were used: ACS grade diethylamine[3], acetonitrile[4] (distilled in glass, UV), and chloroform[4] (distilled in glass, without ethanol preservative).

Apparatus—A high-pressure pump[5] equipped with a 25-μl sample loop injection valve[6] and a multiple-wavelength detector[7] set at 254 nm and attached to a recorder[8] were used.

Column—The column[9] (4.6 mm i.d. × 25 cm) was purchased and used as received. It consisted of porous spherical silica microparticles (5–6 μm).

Mobile Phase Preparation—The mobile phase was prepared by mixing 750 ml of acetonitrile, 250 ml of chloroform, and 0.2 ml of diethylamine. The mobile phase was vacuum filtered through a polytef filter[10] and degassed prior to use.

Conditions for Chromatographic Analysis—The column was conditioned for 16 hr with the mobile phase at a flow rate of 0.2 ml/min. The approximate chromatographic conditions used were a flow rate of 0.7 ml/min (inlet pressure of 400 psig), a chart speed of 0.5 cm/min, and a detector sensitivity of 0.2 absorbance unit full scale (aufs). The column temperature was maintained at 28° by a water jacket controlled by a thermostat.

Standard Solutions of Doxepin Hydrochloride Isomers—Standard solutions of (Z)-doxepin hydrochloride in mobile phase contained 0.007–0.040 mg/ml. (E)-Doxepin hydrochloride standard solutions contained 0.035–0.070 mg/ml. With these (Z)- and (E)-doxepin hydrochloride standard solutions, five (Z)/(E)-doxepin hydrochloride mixtures of known concentrations (Table I) were prepared.

Calibration Curves and Mixture Analysis—Calibration curves were prepared by injecting aliquots (25 μl) of the individual (Z)- and (E)-doxepin hydrochloride standard solutions into the chromatographic system. The peak heights (centimeters) were plotted against the isomer concentrations (milligrams per milliliter), and the slopes of the curves were calculated.

The (Z)- and (E)- content of the five *cis/trans*-mixtures was determined by injecting 25-μl aliquots and measuring the resulting isomer peak heights. The following formulas were used to calculate the percentage of (Z) and (E)-doxepin hydrochloride in each mixture:

$$\% (Z)\text{-isomer} = \frac{H_r}{C} \times \frac{100}{S_Z} \quad \text{(Eq. 1)}$$

$$\% (E)\text{-isomer} = \frac{H_e}{C} \times \frac{100}{S_E} \quad \text{(Eq. 2)}$$

where H_r and H_e are the heights of the (Z)- and (E)-isomer peaks, respectively; C is the concentration (milligrams per milliliter) of both isomers in the mixture, and S_Z and S_E are the slopes of the (Z)- and (E)-isomer calibration curves, respectively. Once the linearity was proven, one (Z)- and (E)-doxepin standard solution was sufficient to determine accurately the isomeric content of doxepin hydrochloride samples.

RESULTS AND DISCUSSION

A typical chromatogram of a (Z)- and (E)-doxepin hydrochloride sample is shown in Fig. 1. Figure 2 illustrates that baseline resolution is easily attainable for (Z)- and (E)-doxepin hydrochloride mixtures of widely varying compositions.

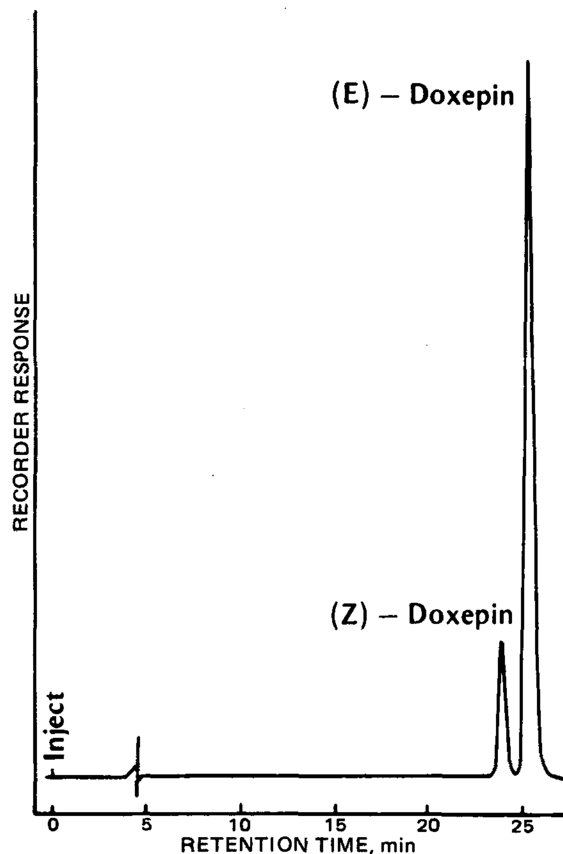


Figure 1—*Typical HPLC chromatogram of doxepin hydrochloride.*

[1] The published HPLC method was originally developed in the Laboratories of Central Research and Quality Control, Pfizer Inc., Groton, Conn., by Mr. T. J. Toolan and Dr. W. P. Zeronsa.
[2] Pfizer, Inc.
[3] Matheson, Coleman and Bell, Norwood, Ohio.
[4] Burdick & Jackson Laboratories, Muskegon, Mich.
[5] Model M6000A, Waters Associates, Milford, Mass.
[6] Valco model CV-UHPa-C-20, 7000-psi pressure rating, distributed by Alltech Associates, Arlington Heights, Ill.
[7] Spectroflow monitor 770, Schoeffel Instrument Corp., Westwood, N.J.
[8] A-25, Varian Instrument Division, Palo Alto, Calif.
[9] Zorbax SIL, Catalog No. 850952702, Du Pont Instruments, Wilmington, Del.
[10] Millipore type LS, 5.0 μm pore, Catalog No. LS (WP) 04700, Millipore Corp., Bedford, Mass.

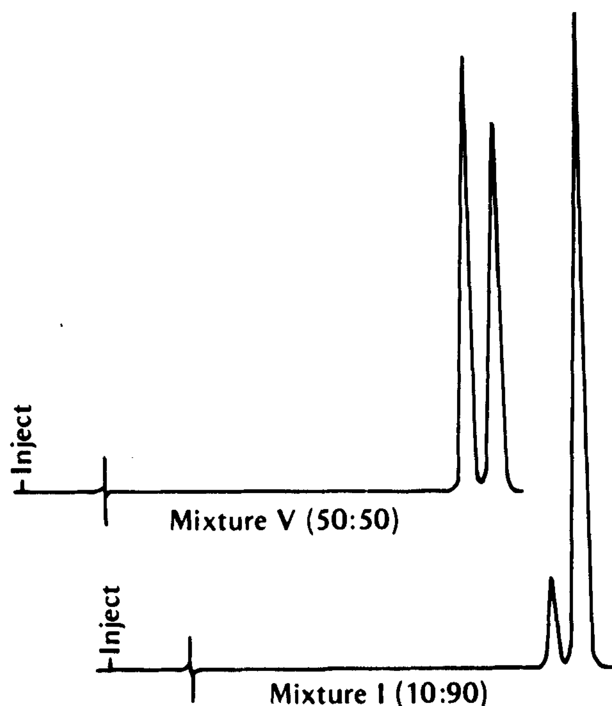


Figure 2—*HPLC chromatograms of mixtures of* (Z)- *and* (E)-*doxepin hydrochloride standards* (*Table I*).

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.

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

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.

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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Received February 21, 1979, from the *Drug Metabolism Department, Pfizer Inc., Groton, CT 06340.* Accepted for publication May 8, 1979. *Department of Pharmacology, Thomas Jefferson University, Philadelphia, PA 19107.

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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