SUMMARY

Adrenochrome semicarbazone has been shown to decrease blood loss in normal mice whose tails have been clipped. The decrease in blood loss does not correlate with bleeding time. The bleeding times increased with anticoagulants but initial blood loss did not change significantly.

Heparin and bishydroxycoumarin have been used to deplete or block the blood stream of necessary clotting components and in these animals adrenochrome semicarbazone can be shown to decrease the blood loss when measured to cessation of the initial blood flow. Adrenochrome semicarbazone will not increase rate of survival in animals lacking a necessary amount of clotting components.

The failure of adenosine and adenosine diphosphate, heparin, and bishydroxycoumarin to influence initial blood loss indicates that in the tail vessel of the mouse the primary hemostatic consideration is that of local vasoconstriction. Adrenochrome semicarbazone appears to augment this constrictor effect.

REFERENCES

(1) Roskam, J., "L'Hemostase Spontanee," Masson & Cie Editeor, Paris, France, pp. 63–64, 81–84.
(2) Fisher, R. A., "Statistical Methods for Research Work," Oliver & Boyd, London, England, 1950, p. 122.
(3) Litchfield, J. T., Jr., and Wilcoxon, F., *J. Pharmacol. Exptl. Therap.*, **96**, 99(1949).
(4) Constantinescu, M., Stoian, F., Papa, V., and Copae, L., *Pharmazie*, **16**, 458(1961).
(5) Zucker, M. B., *Am. J. Physiol.*, **148**, 275(1947).
(6) Solandt, D., and Best, C., *Lancet*, **1**, 1042(1942).
(7) Chen, T. L., and Tsai, C., *J. Physiol.*, **107**, 280 (1948).
(8) Brooks, A. M., and Stewart, G. J., *Fed. Proc. Abstr.*, **25**, 2898(1966).
(9) Johnson, S. A., Fredell, L. M., Shepard, J. A., Tebo, T. H., Pedrson, H. J., and VanHorn, D. L., Fourteenth Annual Symposium on Blood, Wayne State University of Medicine, January 1966, p. 6.
(10) Spaet, T. H., *ibid.*, p. 16.
(11) Bambel, D. E., *ibid.*, p. 16.
(12) Haslam, R. J., *ibid.*, p. 10.

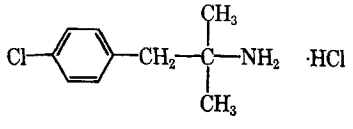
Drug Standards

Qualitative and Quantitative Tests for Chlorphentermine Hydrochloride

By EDWARD F. SALIM*, J. RITNER WEAVER†, and LESTER CHAFETZ†

Provisional, unofficial monographs are developed by the Drug Standards Laboratory, in cooperation with the manufacturers of the drug concerned, for publication in the *Journal of Pharmaceutical Sciences*. The ready availability of this information affords discriminating medical and pharmaceutical practitioners with an added basis for confidence in the quality of new drug products generally, and of those covered by the monographs particularly. Such monographs will appear on drugs representing new chemical entities for which suitable identity tests and assay procedures are not available in the published literature. The purity and assay limits reported for the drugs and their dosage forms are based on observations made on samples representative of commercial production and are considered to be reasonable within expected analytical and manufacturing variation.

p-CHLORO-α,α-DIMETHYLPHENETHYLAMINE HYDROCHLORIDE; $C_{10}H_{14}ClN.HCl$; mol. wt. 220.14. The structural formula of chlorphentermine hydrochloride may be represented as:



Physical Properties—Chlorphentermine hydrochloride occurs as an odorless, white to off-white powder with a bitter taste, m.p. 232–235° (U.S.P. class I). It is freely soluble in water and in alcohol, sparingly soluble in chloroform, and practically insoluble in ether. The pH of a solution of chlorphentermine hydrochloride in carbon dioxide-free water (1 in 100) is between 5.0 and 6.0.

Received August 16, 1966, from the * Drug Standards Laboratory, AMERICAN PHARMACEUTICAL ASSOCIATION FOUNDATION, Washington, DC 20037
Accepted for publication May 10, 1967.
† Warner-Chilcott Laboratories, Morris Plains, NJ 07950.
Warner-Chilcott Laboratories has cooperated by furnishing samples and data to aid in the development and preparation of this monograph.
The Drug Standards Laboratory gratefully acknowledges the assistance of Miss Carolyn Damon and Miss Hannah Klein.

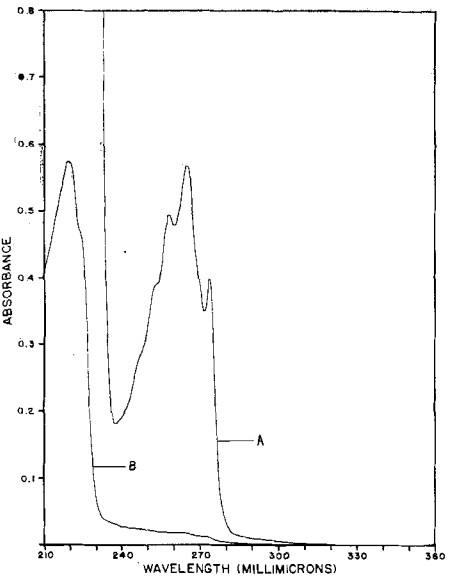


Fig. 1—Ultraviolet absorption spectra of chlorphentermine hydrochloride in water; Beckman model DK-2A spectrophotometer. Key: A, *500 mcg./ml.;* B, *10 mcg./ml.*

Identity Tests—Dissolve about 50 mg. of chlorphentermine hydrochloride in 3 ml. of water, add ammonia T.S. until basic, and filter. Acidify the filtrate with diluted nitric acid and add 1 ml. of silver nitrate T.S.: a white precipitate forms, which is insoluble in diluted nitric acid but soluble in ammonia T.S. (presence of chloride).

A 1 in 2000 solution of chlorphentermine hydrochloride in water exhibits ultraviolet maxima at about 259, 268, and 278 mμ, and absorbance minima at about 239, 261, and 275 mμ. A 1 to 50 dilution of this solution exhibits an absorbance maximum at about 220 mμ [absorptivity (a) about 52]. The spectrum at each concentration is shown in Fig. 1.

The infrared spectrum of a chlorphentermine hydrochloride dispersion in liquid petrolatum is shown in Fig. 2.

Purity Tests—Dry about 1 Gm. of chlorphentermine hydrochloride, accurately weighed, at 105° for 4 hr.: it loses not more than 1% of its weight.

Char about 1 Gm. of chlorphentermine hydrochloride, accurately weighed, ignite, cool, and weigh: the residue does not exceed 0.1%.

Determine the heavy metals content of chlorphentermine hydrochloride by the U.S.P. heavy metals test, method II: the heavy metals limit for chlorphentermine hydrochloride is 20 p.p.m.

Assay—Transfer about 400 mg. of chlorphentermine hydrochloride, accurately weighed, to a tall-form 200-ml. beaker, and add 25 ml. of glacial acetic acid and 10 ml. of mercuric acetate T.S. Warm slightly to effect solution, cool, add 2 drops of crystal violet T.S., and titrate with 0.1 *N* acetous perchloric acid to a blue-green end point. Perform a blank determination, and make any necessary correction. Each milliliter of 0.1 *N* perchloric acid is equivalent to 22.01 mg. of $C_{10}H_{14}ClN.HCl$.

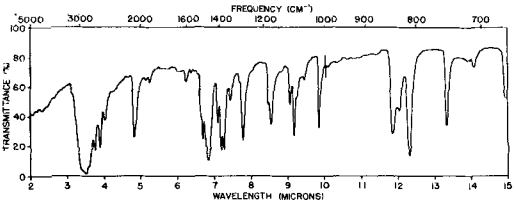


Fig. 2—Infrared spectrum of chlorphentermine hydrochloride dispersion in liquid petrolatum; Perkin-Elmer model 21 spectrophotometer, sodium chloride prism.

The amount of chlorphentermine hydrochloride found is not less than 98.0% and not more than 102.0%.

DOSAGE FORMS OF HYDROCHLORIDE CHLORPHENTERMINE

Chlorphentermine Hydrochloride Tablets

Identity Test—The filtrate from the original tablet solution obtained in the *Assay* exhibits absorbance maxima and minima at the same wavelengths as a solution of chlorphentermine hydrochloride standard in 0.01 *N* hydrochloric acid at a concentration of about 500 mcg./ml.

Assay—Weigh and finely powder not less than 10 chlorphentermine hydrochloride tablets. Weigh accurately a portion of the powder, equivalent to about 500 mg. of chlorphentermine hydrochloride, and transfer to a 1000-ml. volumetric flask. Add about 500 ml. of 0.01 *N* hydrochloric acid and warm on a steam bath for about 15 min. with intermittent agitation. Cool, dilute to volume with 0.01 *N* hydrochloric acid, and mix. Filter the solution through paper discarding the first 50 ml. of filtrate. Transfer 20.0 ml. of clear filtrate into a 125-ml. separator, add 1 ml. of sodium hydroxide T.S., and extract with four 20-ml. portions of ether, collecting the ether extracts in a second separator. Extract the ether phase with two 40-ml. portions of 0.01 *N* hydrochloric acid, combine the acid extracts in a 100-ml. volumetric flask, dilute with the acid to volume, and mix. Dilute 10.0 ml. of this solution to 100.0 ml. with the acid, and mix. Concomitantly determine the absorbance of this solution and of a standard solution of chlorphentermine hydrochloride, in the same medium, at a concentration of about 10 mcg./ml., in 1-cm. cells, at the maximum at about 220 mμ, with a suitable spectrophotometer, using 0.01 *N* hydrochloric acid as the blank. Calculate the quantity, in milligrams, of $C_{10}H_{14}ClN$ in the portion of tablets taken by the formula $50\ C\ (A_u/A_s) \times 0.834$, in which C is the exact concentration of the standard solution, in mcg./ml., A_u is the absorbance of the sample solution, A_s is the absorbance of the chlorphentermine hydrochloride standard solution, and 0.834 is the conversion factor relating chlorphentermine base to chlorphentermine hydrochloride. The amount of chlorphentermine base found is not less than 95.0% and not more than 105.0% of the labeled amount.

DISCUSSION

U.S.P. and N.F. terminology for solubility,

melting range, reagents, *etc.*, has been used wherever feasible.

Chlorphentermine hydrochloride,[1] an anorectic agent indicated for treatment of obesity, is distinguished by a selective pattern of pharmacologic action. This pattern of activity differs qualitatively and quantitatively from the amphetamines, whose indiscriminate effects at anorectic doses on the cardiovascular system and central nervous system are well known. Unlike the amphetamines, chlorphentermine hydrochloride exhibits no adverse effect on blood pressure, heart rate, or blood sugar.

The tablet formulation is a sustained action product so prepared that the medication is released gradually and without interruption over an 8-hr. period. The tablets are labeled to contain 65 mg. of chlorphentermine base which is equivalent to 78 mg. of the hydrochloride salt.

Identity Tests—The chloride test and ultraviolet absorption spectrum are not sufficient for differentiation of chlorphentermine hydrochloride from other sympathomimetic amine hydrochlorides. However, this information together with the infrared spectrum and melting range for chlorphentermine hydrochloride does provide a satisfactory identification. Mason *et al.* (1) have published a comprehensive report dealing with the physical properties of the drug.

Quantitative Methods—The nonaqueous titration for chlorphentermine hydrochloride with perchloric acid using crystal violet T.S. gave an average value of 99.9 ± 0.2%.[2] Analysis of commercial chlorphentermine hydrochloride tablets by the spectrophotometric method gave an average value of 100.5 ± 1.5%.[2] The suitability of the procedure was verified by an average recovery of 100.2 ± 0.3%[2] for a standard chlorphentermine hydrochloride solution carried through the extractive steps as included for the tablets.

[1] Marketed as Pre-Sate by Warner-Chilcott Laboratories, Division of Warner-Lambert Pharmaceutical Co., Inc., Morris Plains, N. J.

REFERENCE

(1) Mason, M. F., Foerster, E., Patterson, W., and Drummon, W., *J. Forensic Sci.*, 11, 243(1966).

[2] Maximum deviation from the mean value.

Notes

Novel Disubstituted Mannich Product as a Potential Anti-Infective Agent

By ROBERT A. MAGARIAN and W. LEWIS NOBLES

Identification of α,α′-di-{3-(3-azabicyclo[3.2.2]-nonyl)-methyl}-*p*-nitroacetophenone is reported.

THE ISOLATION of a new disubstituted Mannich product was mentioned in a previous publication (1). The Mannich reaction, involving the reactive hydrogen compound acetophenone (I), is shown and illustrates the replacement of one of the active hydrogen atoms by an aminomethyl group which may arise from formaldehyde and the amine.

The Mannich product from a methyl ketone (II) contains labile hydrogens, and, in some cases, it is possible to proceed a step further yielding a compound with two basic groups (III) (2).

The amine hydrochloride is usually employed to provide the proper acidic environment. Cummings and Shelton (3) and Hellmann and Opitz (4) have carried out kinetic studies of the Mannich reaction and have concluded that the active aminomethylating agent is the electrophilic carbonium-immonium ion ($R_2\overset{+}{N}{-}\overset{+}{C}H_2 \leftrightarrow R_2\overset{+}{N}{=}CH_2$) which arises only under acidic conditions from the aminomethylol (*N*-hydroxymethylamine) derivative R_2NCH_2OH. The nucleophile is thought to be the enolic tautomer of the ketone which condenses with the aminomethylating agent.

When the free amine is used in equimolar amounts with the other reactants, a dibasic Mannich product may be isolated. The mechanism of the Mannich reaction has not been completely elucidated; it is considered unlikely that a single mechanism can be postulated which will include all known cases involving this reaction. In these studies, the Mannich condensation involving equimolar amounts of *p*-nitroacetophenone, 37% aqueous formaldehyde, and 3-azabicyclo[3.2.2]nonane was first conducted by modifying the conditions set forth by Blanton and Nobles (5); that is, the free base rather than the hydrochloride salt of the amine was employed.

Received December 29, 1967, from the Department of Pharmaceutical Chemistry, School of Pharmacy, The University of Mississippi, University, MS 38677
Accepted for publication May 17, 1967.